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by

MARGARET JEAN PYBUS

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Abstract

The specificity of host-parasite relationships was investigated using *Parelaphostrongylus andersoni* Prestwood 1972 and *P. odocoilei* (Hobmaier and Hobmaier 1934) (Nematoda: Protostrongylidae) in white-tailed deer (*Odocoileus virginianus*) and mule deer (*O. h. hemionus*). The basis used for evaluating the relationships was the premise that well-adapted relationships involve successful establishment and productivity of the parasite in conjunction with a minimum of interference with the host.

Hand-raised fawns approximately four months old were each exposed *per os* to either 300 or 1000 third-stage larvae and were then killed 46 to 151 days after exposure. Establishment (the number of worms recovered as a per cent of the initial dose), productivity, and pathogenicity of infections was determined for each host-parasite relationship.

Parelaphostrongylus andersoni appeared to be better adapted to white-tailed deer than to mule deer. The establishment rate was slightly higher in mule deer, yet the parasites were associated with more damage to the skeletal muscles, lungs, and lymph nodes of the host. Individual variation in infections was more noticeable in white-tailed deer than in mule deer.

Parelaphostrongylus odocoilei was poorly adapted to both mule deer and white-tailed deer. Results were consistent within host species but markedly different

between host species. In mule deer exposed to *P. odocoilei*, a high percentage of the initial dose was recovered as adult worms and larval output was extremely high. However, damage to the host tissues was extensive and severe. In white-tailed deer exposed to *P. odocoilei*, no adult worms were recovered and larval output was low, erratic, and of short duration. This suggested that few worms were able to reach maturity. Damage to white-tailed deer was minimal.

Location, orientation, dispersal, and sex ratio of adult worms within the host was generally similar in all relationships (except that of *P. odocoilei* and white-tailed deer). Generally, the location of adult worms in skeletal muscles was correlated with time after exposure and number of worms recovered but not with dosage level. Adult worms were always in connective tissue, most often coiled between muscle bundles. The sex ratio was consistently 3 females: 2 males.

Response of the host appeared to play the major role in determining the host-parasite relationship. It is suggested that white-tailed deer may be 'pre-adapted' to dealing with infections of *Parelaphostrongylus* spp. and, thus, were able to restrict the infection to moderate or low levels (as seen in fawns exposed to *P. andersoni* or *P. odocoilei*, respectively). Mule deer were less able to control infections and, thus, damage to the host was high. A general discussion of host specificity is included.

Acknowledgements

'With so many people to thank,
one scarcely knows where to begin'

This program could not have been completed without tremendous diligence and effort by many people. Specific agencies and personnel directly involved in different aspects of the work are acknowledged in each chapter of the thesis. A special acknowledgement is given to the Canadian National Sportsmen's Show for financial support throughout much of my Masters and Ph.D. programs. I shall endeavor to show that this confidence was not misplaced.

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I owe Bill Samuel a great deal for his guidance and encouragement throughout this program. Time spent in a graduate program is a unique opportunity for personal as well as scientific development. Through the highs (eg. recovery of a previously unknown worm) and the lows (eg. quarantine at the barn) Bill allowed me the freedom to develop my own ideals and opinions. In all sincerity, Bill, I appreciate the personal and financial resources you made available to me throughout this project (and the others you let me work on). Such debts I can never repay.

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Table of Contents

Chapter — Page

I. Introduction 1

II. Growth of captive white-tailed deer and mule deer fawns 12

- Abstract 12
- Introduction 12
- Materials and Methods 14
- Results 17
- Discussion 19
- Acknowledgements 21
- Literature Cited 21

III. Aspects of the host-parasite relationship of *Parelaphostrongylus andersoni* and *P. odocoilei* within the definitive host 23

- Abstract 23
- Introduction 24
- Materials and Methods 26
- Results 29
- Discussion 46
- Acknowledgements 53
- Literature Cited 54

IV. Pathology of *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) in two cervid hosts ..57

- Abstract 57
- Introduction 58
- Materials and Methods 59

Results 65
Discussion 86
Acknowledgements 93
Literature Cited 94
V. Pathology of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in two cervid hosts ..97
Abstract 97
Introduction 97
Materials and Methods 99
Results 101
Discussion 119
Acknowledgements 122
Literature Cited 123
VI. Concluding Discussion 125
Literature Cited 137
VII. Appendix i: Pathology of the muscleworm, *P. odocoilei* (Nematoda: Metastrongyloidea), in moose..140
VIII. Appendix II: Natural infections of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in several hosts and locations..158
IX. Appendix III: Attempts to find a laboratory host for *Parelaphostrongylus andersoni* and *P. odocoilei* (Nematoda: Protostrongylidae) 167
X. Appendix IV: A case report of two mule deer fawns exposed to 20 third-stage larvae of *P. odocoilei* ..182

List of Tables

Table — Page

Chapter 1

1. Suggested feeding regimen for raising captive deer fawns 15

2. Composition of milk and pelleted rations fed to white-tailed deer and mule deer fawns 16

3. Weight (kg), weight gain (g), and milk consumption (l) of deer fawns received 6-10 June, 1978-1981 18

Chapter 2

1. Experimental design for exposure of fawns to *Parelaphostrongylus* spp. 27

2. Location of adult *Parelaphostrongylus* spp. in the skeletal muscles of white-tailed deer (WTD) and mule deer (MD) 30

3. Groupings of males and females of *Parelaphostrongylus* spp. within white-tailed (WTD) and mule deer (MD) 38

4. First-stage larvae of *Parelaphostrongylus* spp. in experimentally-infected white-tailed deer (WTD) and mule deer (MD) 40

Chapter 3

1. Experimental design to investigate the pathology of *Parelaphostrongylus andersoni* in fawns 61

2. Histopathology of lungs of white-tailed deer (WTD) and mule deer (MD) exposed to *Parelaphostongylus andersoni* 75

3. Quantitative histopathology of lungs of white-tailed deer (WTD) and mule deer (MD) fawns exposed to *Parelaphostongylus andersoni* and of control animals .. 77

4. Histopathology of lymph nodes and spleen of control animals and fawns exposed to *Parelaphostrongylus andersoni* 82

Chapter 4

1. Experimental design to investigate the pathology of *Parelaphostrongyls odocoilei* in fawns 100

2. Haematologic values of fawns exposed to *Parelaphostrongylus odocoilei* and of control animals 103

3. Quantitative histopathology of lungs of white-tailed deer (WTD) and mule deer (MD) fawns exposed to *Parelaphostrongylus odocoilei* and of control animals 116

4. Histopathology of lymph nodes and spleen of control animals and of fawns exposed to *Parelaphostrongylus odocoilei* 117

Discussion

1. The relationship of *Parelaphostrongylus odocoilei* and three cervid hosts 132

Appendix I

1. Infection data for *Parelaphostrongylus odocoilei* in moose 147

Appendix II

1. Selected measurements (um) of adult *Parelaphostrongylus odocoilei* from various hosts 164

Appendix III

1. Recovery of larvae from guinea pigs exposed to *Parelaphostrongylus* spp. 170

2. Recovery of encapsulated larvae (as % per individual host) from rabbits exposed to *Parelaphostrongylus* spp. 172

Appendix IV

1. Location of adult *Parelaphostrongylus odocoilei* in the skeletal muscles of two mule deer (MD) fawns. ...185

List of Figures

Figure — Page

Chapter 1

1. Life cycle of *Parelaphostrongylus* spp. 7

Chapter 2

1. Distribution of adult *Parelaphostrongylus* spp. in white-tailed deer and mule deer 33
2. Adult female *Parelaphostrongylus odocoilei* in intermuscular connective tissue in the abdominal wall 36
3. Adult female *Parelaphostrongylus andersoni* within a vein in the longissimus dorsi 36
4. Number of first-stage larvae in faeces of deer exposed to 300 or 1000 third-stage larvae of *Parelaphostrongylus andersoni* 43
5. Number of first-stage larvae in faeces of deer exposed to 300 or 1000 third-stage larvae of *Parelaphostrongylus odocoilei* 45

Chapter 3

1. Gross haemorrhage in the psoas muscle of a MD exposed to *Parelaphostrongylus andersoni* 69
2. Myositis associated with eggs of *Parelaphostrongylus andersoni* deposited within the biceps femoris of a WTD 69
3. Localized necrosis and diffuse tissue degeneration associated with degenerate eggs of *Parelaphostrongylus andersoni* in the latissimus dorsi of a WTD 69
4. Venous thrombus containing eggs of *Parelaphostrongylus andersoni* and inflammatory cells in the semimembranosus of a MD 69
5. Emphysema and loss of functional lung tissue due to granulomas around eggs and larvae of *Parelaphostrongylus andersoni* 73
6. Necrosis of pulmonary granulomas in a MD 73
7. Accumulation of eggs of *Parelaphostrongylus andersoni* within dilated pulmonary vessels 73

8. Development to larvated eggs of *Parelaphostrongylus anderson*i within dilated blood vessels 73

9. Thrombus of eggs of *Parelaphostrongylus anderson*i and inflammatory cells in an afferent lymphatic of a WTD 80

10. An afferent lymphatic in WTD 41 occluded with larvae of *Parelaphostrongylus anderson*i 80

11. Granulomas associated with eggs of *Parelaphostrongylus anderson*i in the subcapsular sinus in a lymph node of a MD 80

12. Granulomas associated with eggs of *Parelaphostrongylus anderson*i in the paracortical region in a lymph node of a MD 80

Chapter 4

1. Minor haemorrhage and cellular response to adult *Parelaphostrongylus odocoilei* in the biceps femoris of a MD 107

2. Vasculitis associated with a female *Parelaphostrongylus odocoilei* in a vein in the longissimus dorsi of a MD 107

3. Cross-section of an adult *Parelaphostrongylus odocoilei* in the abdominal wall of a MD 107

4. Extensive haemorrhage associated with an adult *Parelaphostrongylus odocoilei* in the longissimus dorsi of a MD 107

5. Granulomatous inflammation associated with eggs and larvae of *Parelaphostrongylus odocoilei* in the fat of a MD 109

6. Granulomatous inflammation associated with eggs and larvae of *Parelaphostrongylus odocoilei* in the gluteus major of a MD 109

7. Larvated eggs of *Parelaphostrongylus odocoilei* in the lung of a MD 109

8. Granulomas associated with eggs and larvae of *Parelaphostrongylus odocoilei* in the lungs of a MD ..109

9. Small discrete granulomas distributed throughout the lung of a MD exposed to *Parelaphostrongylus odocoilei* 113

10. Moderate-sized granulomas associated with

atelectasis and emphysema in a MD exposed to *Parelaphostrongylus odocoilei* 113

11. Large, confluent granulomas associated with extensive atelectasis, haemorrhage, and oedema in a MD exposed to *Parelaphostrongylus odocoilei* 113

12. Lungs of a control animal. 113

Appendix I

1. Life cycle of *Parelaphostrongylus odocoilei* in moose 144

I. Introduction

'Parasitologists have discovered the exceedingly interesting fact that many parasites are strictly host specific' .....Mayr 1957

Host specificity, the ability of a parasite to be successful in one host and not another, is an underlying principle of parasitological theory. Why does a parasite exist as a relatively benign companion in some hosts and as a pathologic insult in others? Interest in such a basic principle has generated a large volume of literature; yet, it remains difficult to generalize about the causes of specificity (Kennedy 1975, Levine 1980). Thus, such questions as where specificity occurs and how it is mediated largely remain unanswered.

Before proceeding, it is necessary to define the scope of this thesis. Since definitions and terminology concerning host specificity vary widely (see Dogiel 1964, Odening 1976), it is not the intent of this thesis to try to review these topics. I shall restrict the topic to only two separate approaches to host specificity currently used by parasitologists. Regardless of approach, some of the criteria used to assess a host-parasite relationship are the same. Using these criteria, I shall present a comparative evaluation of some host-parasite relationships of two closely-related parasite species in two closely-related host species. A comparison of these relationships may provide insight into the mechanisms of specificity.

The traditional definition of specificity in host-parasite relationships is 'the adaptability of a species of parasite to a certain species or group of hosts' (Cheng, 1973. p30). The usual approach to an investigation of host specificity is to assess potential relationships using experimental infections of various host species with the objectives of determining the range of hosts which potentially allow development of the parasite.

A different approach is to evaluate the broader ecological aspects of the host-parasite relationship. Holmes (1976) evaluates relationships in terms of the maintenance of parasite populations. Thus, the actual (rather than potential) role of the host under natural conditions is assessed. Holmes prefers to discuss 'suitability' of a host species for allowing sufficient establishment and reproduction to ensure continuation of the parasite species. Kennedy (1975) also views host-parasite relationships in an ecological sense and discusses the ability of a parasite to establish a balanced, stable system within its host. Presumably, the balanced system would have components of establishment and reproductive success resulting in continuation of the parasite species. The objectives of these studies are to evaluate what portion of the total successful reproduction of the parasite is contributed by individuals in different host species.

As listed by Odening (1976), the criteria used to evaluate host-parasite relationships include optimum chance

of the host and parasite meeting, prevalence and intensity in the host species, rate of parasite development, longevity in the host, parasite reproductive capacity, intensity of the host defense response, ability of the host to withstand pathologic insult, viability and transmissive potential of the parasite reproductive products, and continuation of the above features over numerous host passages. Some of these criteria pertain to both approaches mentioned above, while others are restricted by methodology to one or the other.

The above criteria will be used, where applicable, to evaluate the host-parasite relationships between each of two metastrongyloid nematodes and two cervid definitive hosts. The relationships are compared in terms of the suitability and stability of the systems. Data from infections in various other hosts are included in an overall discussion of host specificity.

General information

Nematodes of the superfamily Metastrongyloidea are a relatively homogeneous group of endoparasites associated with the respiratory system of mammals at some stage in the life cycle. They have a heteroxenous life cycle using gastropods (or, rarely, oligochaetes) as intermediate hosts. Metastrongyles are considered to have a fairly narrow host specificity range (Anderson 1971, p83); however, it is often unknown where or how this specificity is mediated.

Theoretically, specificity may occur at any time during the life cycle. However, Dogiel (1964) suggests that host specificity is more restricted in situations when the parasite undergoes major physiologic and morphologic changes. Within the intermediate hosts, metastrongyles develop from first to third-stage larvae with very little change in size and morphology. Specificity in intermediate hosts is often low (see Mackerras and Sandars 1955, Panin 1967, for example) and does not appear to account for the limited specificity in the overall life cycle.

Transmission of metastrongyles to the final host often involves accidental ingestion of infected snails (by herbivores) or ingestion of paratenic hosts (by carnivores). Thus, the potential for transmission of infective larvae to the final host should be similar for all direct 'ecological equivalents'. If all such hosts are exposed, any differences in the host-parasite relationship would appear to be mediated within the final host.

Nematodes of the genus *Parelaphostrongylus* (Metastrongyloidea: Protostrongylidae) are parasites common in cervids and, perhaps, bovids in North America. They are of concern in free-ranging populations of cervids due to their pathologic effects on a variety of wild and domestic hosts. The genus currently contains three species: *P. andersoni* Prestwood 1972, *P. odocoilei* (Hobmaier and Hobmaier 1934), and *P. tenuis* (Dougherty 1945).

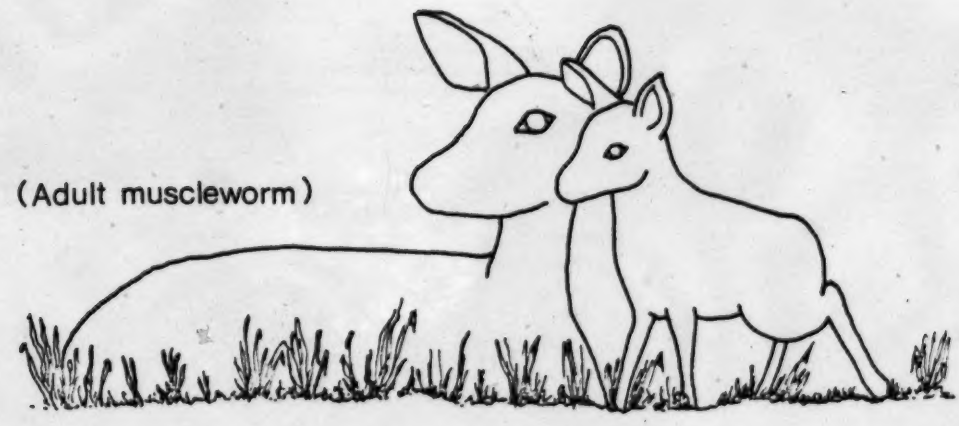
The life cycle of *Parelaphostrongylus* spp. (see Fig. 1 and Anderson 1971) is as follows: Adult worms mature in the definitive host. Eggs are released from females into the venous blood system and eventually lodge in capillary beds of the lungs. First-stage larvae pass up the trachea, enter the oesophagus and leave the intestine with the faeces. Larvae subsequently penetrate the foot of a wide variety of terrestrial gastropods and develop to infective third-stage larvae. These molluscs are then accidentally ingested by grazing or browsing cervids. Larvae are digested from the gastropod tissue and migrate to the preferred site for maturity.

Current information about *P. andersoni* concerns its taxonomic designation (Prestwood 1972), geographic distribution (Prestwood et al. 1974, Pursglove 1977, Pybus and Samuel 1981), and pathologic effects in experimentally-infected white-tailed deer (*Odocoileus virginianus*) (WTD) (Nettles and Prestwood 1976, Prestwood and Nettles 1977).

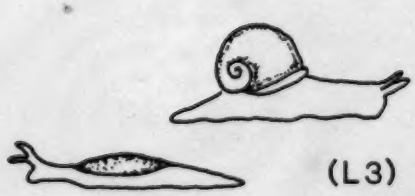
Information about *P. odocoilei* has centered around its taxonomic designation (Hobmaier and Hobmaier 1934, Brunetti 1969, Platt and Samuel 1978a), life cycle in cervid hosts (Platt and Samuel 1978b), and pathogenesis in the lungs of the definitive host (Hobmaier 1937).

Parelaphostrongylus tenuis has been studied extensively in a variety of definitive hosts (see review in Anderson 1971). Development, migration, geographic distribution, and

Figure 1. Life cycle of Parelaphostrongylus spp. (L1 = first-stage larva, L3 = third-stage larva)


(Adult muscleworm)

(L1)


(L3)

pathologic effects have been emphasized.

Specific hypotheses to be tested in this thesis are 1) *Odocoileus hemionus hemionus* (MD) are well-adapted to *Parelaphostrongylus odocoilei*, and 2) *O. virginianus* are well-adapted to *P. andersoni*.

The methods to be used involve: 1) a comparative clinical and pathological study of *P. odocoilei* infections in MD and WTD. Infections in guinea pigs (*Cavia porcellus*), domestic rabbits (*Oryctolagus cuniculus*), goats (*Capra hircus*), and moose (*Alces alces*) will also be evaluated. 2) a comparative clinical and pathological study of *P. andersoni* infections in WTD and MD. Infections in guinea pigs and rabbits will also be evaluated. 3) evaluation of evidence from natural infections of *P. odocoilei* in black-tailed deer (*O. h. columbianus*), mountain goats (*Oreamnos americanus*), and a hybrid WTD/MD.

Chapters within this thesis deal with separate criteria used to evaluate host-parasite relationships. In order to accurately assess an experimental infection, it is important to know the condition of the animals prior to starting the experiment. Thus, in Chapter II, our regimen for raising neonatal fawns for experimental purposes is described and evaluated. In Chapter III, the establishment, distribution, sex ratio, and larval output of adult *P. andersoni* and *P. odocoilei* in WTD and MD are used to compare infectivity and productivity in the relationships. In Chapters IV and V,

pathologic changes associated with *P. andersoni* and *P. odocoilei* infections in WTD and MD, respectively, are described and used to compare the pathogenicity of the infections.

The general discussion evaluates the overall relationship of each parasite with the final hosts and then compares the relationships of *P. andersoni* with those of *P. odocoilei*. A discussion of host specificity and host suitability is included. The results are used to investigate the evolutionary origins of *P. odocoilei*.

Four appendices are included. In Appendix I, the pathology of *P. odocoilei* in two experimentally-infected moose calves is described. In Appendix II, natural infections of *P. odocoilei* in various hosts and locations are reported. In Appendix III, attempts to infect laboratory hosts with *P. andersoni* or *P. odocoilei* are described. In Appendix IV, a case report of low level infections of *P. odocoilei* in two MD fawns is presented.

LITERATURE CITED

Anderson,R.C. 1971. Lungworms. *In*: Parasitic diseases of wild mammals. J.W. Davis and R.C. Anderson (eds.) Iowa State Univ. Press, Ames, Iowa. pp. 81-126.

Brunetti, O.A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game 55: 307-316.

Cheng, T.C. 1973. General Parasitology. Academic Press, New York and London. 965pp.

Dogiel, V.A. 1964. General Parasitology. Oliver and Boyd Ltd., Edinburgh. 516pp.

Hobmaier, A., and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n. sp., a new lungworm in black tail deer (*Odocoileus columbianus*). Description and life history. Proc. Soc. Exp. Biol. Med. 31: 509-514.

Hobmaier, M. 1937. Studies on the pathology of *Elaphostrongylus odocoilei* in *Odocoileus columbianus columbianus*. Raboty Gel'mint pp 235-240.

Holmes, J.C. 1976. Host selection and its consequences. *In*: Ecological aspects of parasitology. C.R. Kennedy (ed.), North-Holland Publ. Co., Amsterdam. pp21-39.

Kennedy, C.R. 1975. Ecological animal parasitology. Blackwell Scientific Publications, Oxford. 163pp.

Levine, N.D. 1980. Nematode parasites of domestic animals and of man. 2nd ed. Burgess Pub. Co., Minneapolis. 477 pp.

Mackerras, M.J. and D.F. Sandars. 1965. The life history of the rat lungworm, *Angiostrongylus cantonensis* (Chen) (Nematoda: Metastrongylidae). Aust. J. Zool. 3: 1-21.

Mayr, E. 1957. Evolutionary aspects of host specificity among parasites of vertebrates. *In*: Premier symposium sur la specificite parasitaire des parasites de vertebres. Union Internationale des Sciences Biologiques, Serie B, No. 32, pp 7-14.

Nettles, V.F. and A.K. Prestwood. 1976. Experimental *Parelaphostrongylus andersoni* infections in white-tailed deer. Vet. Pathol. 13: 381-393.

Odening, K. 1976. Conception and terminology of hosts in parasitology. Adv. in Parasitol. 14: 1-93.

Panin, V.Ya. 1967. Developmental cycle of *Elaphostrongylus panticola*, Lubimov, 1945. U.S. Dept. Commerce, Springfield. [English translation, IPST Cat. No. 1869]

Platt, T.R. and W.M. Samuel. 1978a. A redescription and neotype designation for *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea). J. Parasitol. 64: 226-232.

Platt, T.R., and W.M. Samuel. 1978b. *Parelaphostrongylus odocoilei*: life cycle in experimentally infected cervids including the mule deer, *Odocoileus h. hemionus*. Exp. Parasitol. 46: 330-338.

Prestwood, A.K. 1972. *Parelaphostrongylus andersoni* sp. n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus*

virginianus). J. Parasitol. 58: 897-902.

Prestwood, A.K., V.F. Nettles, and F.E. Kellogg. 1974. Distribution of muscleworm, *Parelaphostrongylus andersoni*, among white-tailed deer of the southeastern United States. J. Wildl. Dis. 10: 404-409.

Prestwood, A.K. and V.F. Nettles. 1977. Repeated low-level infection of white-tailed deer with *Parelaphostrongylus andersoni*. J. Parasitol. 63: 974-978.

Pursglove, S.R. 1977. Helminth parasites of white-tailed deer (*Odocoileus virginianus*) from New Jersey and Oklahoma. Proc. Helminth. Soc. Wash. 44: 107-108.

Pybus, M.J., and W.M. Samuel. 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. 45: 537-542.

II. Growth of captive white-tailed deer and mule deer fawns.

ABSTRACT

Weight gain, as an indicator of growth rate and condition, was evaluated in 14 white-tailed deer (*Odocoileus virginianus*) and 21 mule deer (*O. hemionus*) fawns fed a diet of 2 parts whole milk:1 part evaporated milk supplemented with bovine colostrum, when available, throughout the pre-weaning period. White-tailed deer and mule deer acheived similar weight gains (217±41, 209±36 grams/day, respectively) and final weights (20.4±3, 19.3±3 kg, respectively). Husbandry of neonatal fawns is discussed briefly.

INTRODUCTION

In order to adequately assess the pathological impact of a parasite on its host, it is essential to know the condition of the animal prior to insult. The researcher relies on sound management techniques to provide a healthy, uninfected animal for future experimentation. In our lab, which is involved in parasitological studies of white-tailed deer (*Odocoileus virginianus*) (WTD) and mule deer (*O. hemionus hemionus*) (MD), this requires the hand-rearing of neonatal fawns.

The literature concerning the raising of neonatal deer fawns abounds with methodology. Unfortunately, many reports

are contradictory and no clear preferred method has been established (see Halford and Alldredge 1978). In general, a diet of fortified bovine milk or evaporated milk appears to be most successful for raising deer fawns (Long et al. 1961, Silver 1961). Deer milk is significantly higher in protein and fat than bovine milk (Silver 1961) and as a result, bovine colostrum may be added to the ration to increase the protein content (Winton and Winton 1937) and vitamin A levels (Halford and Alldredge 1978).

When dealing with parasites of large game animals, there is often no shortage of parasites but severely restricted numbers of hosts. As a result, it is often not possible to use postmortem evaluation of a subsample of hosts to assess condition prior to experimentation. In this study, weight gain, as an indicator of growth rate, is used to evaluate pre-exposure condition in WTD and MD.

The objectives of this study are twofold: 1) to determine whether captive fawns raised under the current regimen can attain weight gains similar to those reported for dam-raised fawns and 2) to compare the growth rates of WTD and MD raised under similar conditions. For the analyses, a subset of animals was chosen to allow standardization of age, condition at arrival, and time spent under the regimen in order to allow direct comparison of results. Other deer were maintained on the same regimen starting whenever they were received.

MATERIALS AND METHODS

Fawns were received as orphans at the University of Alberta Biomedical Animal Centre, Ellerslie, Alberta, between 6-10 June, 1978-1981. All animals were received in apparently healthy condition; that is, active, eager to feed, and with no apparent external or internal injuries. Parturition was standardized as June 1 and all animals were considered as initially in their second week.

Fawns were bottle-fed on a prescribed schedule (Table 1) . From 1978-1981, 14 WTD and 21 MD were maintained on a mixture of two parts whole unpasteurized bovine milk to one part evaporated milk (2:1). The formula was supplemented with 59 ml (2 oz) of bovine colostrum, when available, at each feeding from arrival until weaning at approximately 13 weeks of age (August 20-23). Table 2 contains an analysis of the diet.

Dirt and water were available *ad libitum* throughout the pre-weaning period. After approximately 4 weeks, alfalfa hay and fresh aspen browse were also available. To facilitate weaning, fawns were exposed to increasing proportions of rolled oats in dirt and were eventually weaned onto a mixture of commercial deer pellets (Department of Animal Science, University of Alberta) and beet pulp (Table 2). Beet pulp was deleted from the diet after 1978.

Fawns were initially housed inside in wire pens on cement floors with straw bedding. Usually 5, but up to 10, animals were held in a 9 sq. m. pen. After 1-2 weeks, deer

TABLE 1. Suggested feeding regimen for raising captive deer fawns.

Week	No. daily feedings	Formula/ feeding	Daily total(ml)	Feeding schedule
1	6	177 + 59*	1416	0400,0800,1200,1600,2000,2400
2	6	177 + 59	1416	
3	5	236 + 59	1475	0100,0700,1200,1600,2000
4	5	295 + 59	1770	
5	4	413 + 59	1888	0800,1300,1900,2400
6	3	472 + 59	1593	0800,1500,2200
7	3	531 + 59	1770	
8	3	531 + 59	1770	
9	2	679 + 59	1475	0800,2000
10	2	679 + 59	1475	
11	1	531 + 59	590	0800
11½	1	384 + 59	443	
12	w e a n e d			

* No. milliliters 2 parts whole milk:1 part evaporated milk + bovine colostrum.

TABLE 2. Composition of milk and pelleted rations fed to white-tailed deer and mule deer fawns, 1978-1981.

	Milk*	Pellets
Protein (%)	6.2	17.2
Fat (%)	5.8	2.0
Lactose (%)	4.78	
Calcium (%)	0.91	1.11
Phosphorus (%)	0.26	0.46
Iron (PPM)	2.12	trace
Zinc (PPM)	8.24	trace
Vitamin A (I.U./100ml)	499	9.9×10^6**
Vitamin D (I.U./100ml)	12	1.2×10^6**
Fibre (%)		67.2
Moisture (%)		10.4

* 177 ml. of 2 parts whole milk:1 part evaporated milk + 59 ml. bovine colostrum.

** I.U./Kg.

were given access to 6X10 m. outside wire runs with a cement floor and, in 1978-1980, eventual access to a large grassy paddock.

Detailed daily records of activity, food consumption, defecation, and urination were recorded after each feeding. Animals were weighed twice a week. Initial weight, final weight at weaning, and total amount of formula consumed were used to determine weight gain per day and per liter of formula. These data were used to compare growth rates between the two deer species by a Student's T-test. A significance level of 0.05 was accepted.

RESULTS

Table 3 presents weight and consumption data of 14 WTD and 21 MD fawns. Mean initial weight did not differ between deer species ($t=0.93$, $d.f.=33$, $p=0.35$) and final weight achieved at weaning was similar in WTD and MD ($t=1.01$, $d.f.=33$, $p=0.32$).

Although MD consumed significantly less than WTD ($t=3.25$, $d.f.=33$, $p=0.003$), this was not reflected in weight gains per liter of formula ($t=1.38$, $d.f.=33$, $p=0.18$).

In 1978-1981, an additional 40 WTD and 31 MD were received from June 11 to August 6 at the Biomedical Centre and maintained under the management regimen described herein. Thirty seven WTD and 26 MD (93% and 84%, respectively) fed 2:1 plus colostrum survived to weaning.

TABLE 3. Weight (kg), weight gain (g), and milk consumption (l) of deer fawns received 6-10 June, 1978-1981.

	White-tailed deer	Mule deer
Formula	2:1*	2:1
N	14	21
Initial weight	4.7 ± 0.9	4.3 ± 1.2
Weaning weight	20.4 ± 2.8	19.3 ± 3.2
Weight change	16.0 ± 3.0	15.0 ± 2.8
Gain/day	217.4 ± 40.5	208.6 ± 36.1
Consumption	92.4 ± 7.9	81.0 ± 11.5
Gain/liter	172.5 ± 29.5	185.2 ± 24.8

* 2 parts whole milk:1 part evaporated milk + bovine colostrum.

DISCUSSION

Buckland et al. (1975) conclude that husbandry rather than diet is of primary importance in raising deer fawns. To some extent, we agree with this conclusion. It is essential to maintain high standards of cleanliness as well as detailed records of individual deer from time of arrival until weaning. A regular feeding schedule should be outlined in advance and adhered to as much as possible. Changes in feeding schedules must be carried out gradually. The recognition of disease and immediate removal of the affected animal to an isolated area is also essential. New arrivals introduced into the facility should also be isolated for 3-4 days. It is our experience that these techniques in conjunction with even a moderate plane of nutrition can result in acceptable growth and condition.

Diarrhea is one of the major problems encountered in captive deer fawns (French et al. 1956, Murphy 1960, Kramer et al. 1971, Reichert 1972, Buckland et al. 1975, Halford and Alldredge 1978). Halford and Alldredge (1978) suggested that addition of small amounts of bovine colostrum to the diet may impart immunological resistance and result in a reduced incidence of diarrhea in fawns less than 7 days old. French et al. (1956) provided a diet of colostrum milk to successfully treat cases of severe diarrhea. In our experience, addition of bovine colostrum throughout the pre-weaning period reduces the occurrence of intestinal disorders. Diarrhea was observed, particularly in MD, only

during periods when colostrum was not available or when formula changes were being made. The mechanism and mode of action are unknown.

Direct comparison between current weight gains and previous reports are difficult due to variations in diet and handling procedures. In general, current weight gains of WTD are similar to those in previous reports of fawns hand-raised on fortified whole or evaporated milk (Murphy 1960, Thompson et al. 1973). Robbins and Moen (1975) reported greater gains in fawns fed a formula similar to doe's milk. Weight gains in fawns raised by captive does were slightly higher than those in the current study (Murphy and Coates 1966, Robbins and Moen 1975).

Current weight gains of MD fawns compare favourably with previous reports and were similar to those of dam-raised fawns (Nichol 1936, Robinette et al. 1973, Halford and Alldredge 1978).

A comparison of previous work suggests that hand-raised WTD gain weight more quickly than hand-raised MD (see Murphy 1960, Thompson et al. 1973, Robbins and Moen 1975 for WTD and Reichart unpubl. data, Halford and Alldredge 1978 for MD). However, current data indicate that growth rate in the 2 species is similar in fawns on the same plane of nutrition and maintained under similar management regimens. Dam-raised WTD and MD also gain weight at similar rates (Murphy and Coates 1966, Robbins and Moen 1975, and Nichol 1936, Robinette et al. 1973, Halford and Alldredge 1978).

Weight gains similar to those attained by dam-raised animals suggest that the growth rate and condition of fawns raised in the current study approach those of free-ranging animals. Fawns raised under this management regimen should be adequately suited to withstand the rigours of captivity and experimentation and, thus, provide more meaningful experimental results.

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LITERATURE CITED

Buckland, D.E., W.A. Abler, and R.L. Kirkpatrick. 1975.

Improved husbandry system for rearing fawns in captivity. J. Wildl. Manage. 39: 211-214.

French, C.E., L.C. McEwen, N.D. Magruder, R.H. Ingram and R.W. Swift. 1956. Nutrient requirements for growth and antler development in the white-tailed deer. J. Wildl. Manage. 20: 221-232.

Halford, D.K. and A.W. Alldredge. 1978. A method for artificially raising mule deer fawns. Am. Midl. Nat. 100: 493-498.

Kramer, T.T., J.G. Nagy and T.A. Barber. 1971. Diarrhea in captive mule deer fawns attributed to *Escherichia coli*. J. Wildl. Manage. 35: 205-209.

Long, T.A., R.L. Cowan, C.W. Wolfe and R.W. Swift. 1961. Feeding the white-tailed deer fawn. J. Wildl. Manage. 25: 94-95.

Murphy, D.A. 1960. Rearing and breeding white-tailed fawns in captivity. J. Wildl. Manage. 24: 439-441.

Murphy, D.A. and J.A. Coates. 1966. Effects of dietary protein on deer. Proc. N. Amer. Wildl. Conf. 31: 129-138.

Nichol, A.A. 1936. The experimental feeding of deer. Proc. N. Am. Wildl. Conf. 1: 403-410.

Reichert, D.W. 1972. Rearing and training deer for food habits studies. USDA Forest Service Research Note RM-208.

Robbins, C.T. and A.N. Moen. 1975. Milk consumption and weight gain of white-tailed deer. J. Wildl. Manage. 39: 355-360.

Robinette, W.L., C.H. Baer, R.E. Pillmore and C.E. Knittle. 1973. Effects of nutritional change on captive mule deer. J. Wildl. Manage. 37: 312-326.

Silver, H. 1961. Deer milk compared with substitute milk for fawns. J. Wildl. Manage. 25: 66-70.

Thompson, C.B., J.B. Holter, H.H. Hayes, H. Silver and W.E. Urban. 1973. Nutrition of white-tailed deer. 1. Energy requirements of fawns. J. Wildl. Manage. 37: 301-311.

Winton, A.L. and K.B. Winton. 1937. The structure and composition of foods. Vol. 3. John Wiley and Sons, New York.

III. Aspects of the host-parasite relationship of *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) and *P. odocoilei* within the definitive host.

ABSTRACT

The host-parasite relationship of *Parelaphostrongylus andersoni* Prestwood 1972 and *P. odocoilei* (Hobmaier and Hobmaier 1934) was compared using infectivity, location, orientation, and productivity of adult worms within the final host. Fourteen white-tailed deer (*Odocoileus virginianus*) and ten mule deer (*Odocoileus hemionus*) were exposed to third-stage larvae of *P. andersoni* or *P. odocoilei*. Few differences were noted in location and orientation between *P. andersoni* and *P. odocoilei* in their usual hosts (white-tailed deer and mule deer, respectively) or between different dosage levels.

Recovery of adult worms (as percentage of dose per individual deer) was high in six mule deer exposed to *P. odocoilei* ($\bar{x}$=45), moderate in seven white-tailed deer and four mule deer exposed to *P. andersoni* ($\bar{x}$=20 and 31, respectively), and zero in seven white-tailed deer exposed to *P. odocoilei*. Recovery rates were higher than indicated previously. Female/male pairs were the most common worm association; however, Sex ratio of adult worms was consistently 3 females:2 males throughout the infection. Most adult worms (>90%) were located in connective tissue

within skeletal muscles; a few were in connective tissue within the vertebral canal, and, occasionally, in connective tissue of fat deposits. Distribution among muscles varied with time after exposure and total number of worms recovered.

The pattern of larval output in faeces was similar in most infections, except white-tailed deer exposed to *P. odocoilei*, and consisted of an exponential increase in the first two to three weeks of patency followed by a slow increase, peaking at approximately 6 weeks. Output in white-tailed deer exposed to *P. odocoilei* was erratic. Quantitatively, maximum weekly larval output differed between infections and was extremely high in five mule deer exposed to *P. odocoilei* ($\bar{x}$=15,103 larvae/gram [l/gm]), moderate in eight white-tailed deer and three mule deer exposed to *P. andersoni* ($\bar{x}$=1,834 and 2,630, respectively), and extremely low in seven white-tailed deer exposed to *P. odocoilei* ($\bar{x}$=0.9).

The host-parasite relationships were similar in WTD and MD exposed to *P. andersoni*. In contrast, the relationships of *P. odocoilei* and the hosts differed markedly.

INTRODUCTION

Nematodes of the genus *Parelaphostrongylus* Boev and Schuls 1950 and the closely-related *Elaphostrongylus* Cameron 1931 are parasites found commonly in the central nervous system (CNS) and skeletal muscles of a variety of ruminant

hosts. The species involved are *P. andersoni* Prestwood 1972, *P. odocoilei* (Hobmaier and Hobmaier 1934), *P. tenuis* (Dougherty 1945), and *E. cervi* Cameron 1931. In previous reports, the geographic distribution, development and pathology in the final host, and spectrum of natural and potential hosts have been emphasized (see Anderson 1971, Anderson and Prestwood 1981, for review).

The host-parasite relationship of the species inhabiting skeletal muscles is poorly understood. Adult *P. andersoni* are reported from the dorsal posterior muscles of experimentally-infected (Prestwood 1972, Nettles and Prestwood 1976, Prestwood and Nettles 1977, Pybus and Samuel 1981) and free-ranging (Prestwood et el. 1974) white-tailed deer (*Odocoileus virginianus*) (WTD). Adult *P. odocoilei* are reported from the lymphatic spaces and connective tissue of muscles and vessels of the hind legs of black-tailed deer (*O. h. columbianus*) (Hobmaier and Hobmaier 1934) and the skeletal muscles posterior to the shoulders of mule deer (*O. h. hemionus*) (MD) (Brunetti 1969, Platt and Samuel 1978a). The infectivity of these worms for the definitive host has not been investigated.

Parelaphostrongylus andersoni and *P. odocoilei* appear to be similar parasites using different hosts. In order to compare the host-parasite relationship of these worms in the definitive host, the precise location within the host and the ability to establish an infection was investigated. A series of experimental infections using WTD, MD, *P.*

andersoni, and *P. odocoilei* was conducted. This paper compares the establishment rates, location and orientation of adult worms within the final host, and associations between males and females of each nematode species. The location and number of first-stage larvae produced is used to compare the productivity of the two worm species.

MATERIALS AND METHODS

Neonatal white-tailed deer and mule deer were collected and maintained as outlined in Chapter II. At approximately 4 months of age, fawns were exposed *per os* to third-stage larvae of either *Parelaphostrongylus andersoni* or *P. odocoilei* (Table 1). The larvae were suspended in a syringe containing approximately 2cc of physiological saline and flushed into the back of each animal's throat. The syringe was then rinsed twice with saline and the washings also administered to the deer. Daily faecal samples were monitored by the Baermann technique (as in Platt and Samuel 1978a) from 40 d post exposure (dPE) until termination of the animals. Data are presented as mean weekly larval output throughout patency.

Animals were killed at different times after exposure to larvae by injection of sodium pentabarbitol into a jugular vein. Within 30 minutes after death, various tissue samples were fixed in 10% buffered formalin for later histologic examination. Individual skeletal muscles were examined for nematodes. Backstrap muscles (longissimus

TABLE 1. Experimental design for exposure of fawns to *Parelaphostrongylus* spp.

Year	Worm species	Dose	White-tailed deer	Mule deer
1978				
	P. andersoni	388	WTD 33	
	P. odocoilei	300	WTD 28, 29	MD 14, 20, 21
	control		WTD 26, 30	
1979				
	P. andersoni	300	WTD 38, 39, 42	MD 25, 27
		1000	WTD 41, 51	
	P. odocoilei	300	WTD 37, 45, 50	MD 26
		1000	WTD 44, 46	
	control		WTD 40, 43	MD 30
1980				
	P. andersoni	300	WTD 63	MD 38, 39
	control		WTD 59, 62, 66	MD 31, 32, 33, 34, 36
1981				
	P. odocoilei	300		MD 41, 55
	control			MD 40, 44, 45, 48, 51

dorsi, psoas, and iliacus) were teased apart and examined at 6X magnification. Remaining muscles were sliced at 3-5mm and examined grossly for worms and/or haemorrhagic areas. Such areas were then dissected at 6X magnification. Connective and nervous tissues within skeletal musculature or associated fat were also examined.

Standard procedures were used to examine internal organs and the gastro-intestinal tract for helminths and/or pathologic lesions. However, specific attention was given to the lungs and spinal cord. Total weight of the lungs was recorded and approximately 200g from throughout the lungs was placed in pepsin-HCl digest (0.6g pepsin, 0.7ml HCl in 100ml distilled water) at 37C for at least 12h. The spinal cord was collected, the dura mater removed, and both tissues were examined for helminths at 6X magnification. In five animals, the vertebral canal and connective tissue of the pelvic region were examined in detail. If no worms were recovered from muscles, the brain was split sagittally and the brain and cranial cavity examined at 6X magnification.

In 1978 and 1979, control animals were treated and examined as above excluding exposure to nematodes. In 1980 and 1981, ten MD and three WTD were monitored as controls for first-stage larvae in faeces. These animals were subsequently used in other experiments.

Since methods were not similar in all deer, the worms recovered from the vertebral canal and pelvic regions were not included in the analyses of total worm recovery. Worm

recovery (=establishment rate) was expressed as a percentage of the initial dose. The location of adult worms in various body regions was expressed as a percentage of the total number of worms recovered from each deer. Sex and orientation of worms within tissues were identified where possible and the former, analyzed by male/female associations. Sex data were combined by parasite species and tested by analysis of variance followed by pairwise comparison of group means. A significance level of 0.05 was accepted. Some missing data were unavoidable and sample sizes are indicated for each analysis.

RESULTS

The establishment rate in the two hosts was similar in *P. andersoni* infections but markedly different in *P. odocoilei* infections. Mean worm recovery in five WTD and four MD exposed to 300 (one WTD received 388) third-stage larvae of *P. andersoni* was 15±12 and 31±13%, respectively, while recovery from two WTD exposed to 1000 larvae was 47 and 21%. Adult worms were recovered from all animals exposed to *P. andersoni*. Mean worm recovery in six MD exposed to 300 larvae of *P. odocoilei* was 45±8% but no adult worms were recovered from seven WTD fawns exposed to either 300 or 1000 larvae of *P. odocoilei*. Worms were not found in control animals.

Adult *P. andersoni* and *P. odocoilei* were each recovered from a variety of skeletal muscles (Table 2), and few

TABLE 2. Location of adult Parelaphostrongylus spp. in the skeletal muscles of white-tailed deer (WTD) and mule deer (MD).

	Days post exposure	Total recovery		Regional recovery							
		No.	% dose	Bk	Th	Abd	Sh	Sk	Fo	In	N
MD 27[a]	46	56	19	95	5	0	0	0	0	0	0
MD 55[o]	54	163	54	30	41	9	18	1	1	0	0
WTD 41[a]	79	467	47	47	28	6	4	0	2	1	0
MD 38[a]	82	118	39	85	7	8	0	0	0	0	0
WTD 38[a]	86	12	4	83	17	0	0	0	0	0	0
WTD 42[a]	90	55	18	53	47	0	0	0	0	0	0
WTD 33[a]	98	116	30	72	28	-	0	0	0	-	-
MD 21[o]	99	112	38	35	50	10	4	0	0	1	0
WTD 39[a]	100	60	20	38	48	0	10	0	3	0	0
MD 39[a]	104	64	21	63	34	3	0	0	0	0	0
MD 14[o]	104	152	51	27	51	10	12	0	0	1	0
WTD 51[a]	104	216	21	22	47	7	9	4	1	0	1
WTD 63[a]	108	4	1	25	75	0	0	0	0	0	0
MD 26[o]	121	159	53	18	47	12	11	6	5	0	2
MD 41[o]	126	114	38	25	50	8	6	4	0	0	0
MD 25[a]	138	134	45	51	36	9	4	0	0	0	0
MD 20[o]	138	113	38	19	49	10	16	4	0	1	0

Bk=Backstraps; Th=Thighs; Abd=Abdominal wall; Sh=Shoulder; Sk=Shank; Fo=Forearm; In=Intercostals; N=Neck.

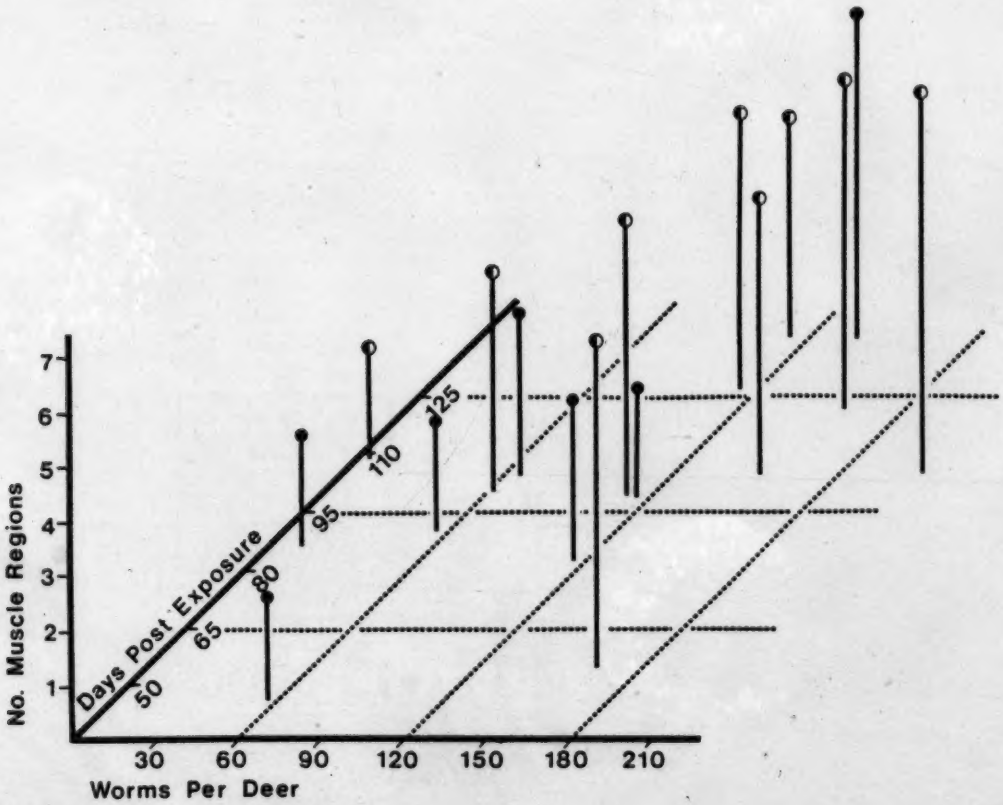
a - P. andersoni; o - P. odocoilei

differences were noted regardless of the host. All infected deer (except WTD exposed to *P. odocoilei*) harbored worms in the backstraps and thighs. Distribution of worms within the carcass appeared to be related to the time after exposure and the total number of worms recovered (Fig. 1, Table 2). Early in the infection (<100 dPE), the majority of the worms was in the backstraps (five of eight deer). Four of seven deer had worms only in the backstraps and thighs. After 100 dPE, only two of nine deer had a majority of the worms in the backstraps and only one fawn had worms restricted to the backstraps and thighs.

The number of muscle regions containing adult worms increased throughout the infection (Fig. 1). More specifically, worms appeared to disperse from the backstraps into the thighs, abdominal wall, thorax, lower legs, and neck. Worm location in the host also was related to the total number of worms in the carcass. Adult worms were restricted to the backstraps and thighs in four of six deer which harbored less than 100 adult worms but were more widely distributed in 10 of 11 deer which harbored more than 100 worms. Distribution of worms in the carcass was not correlated with the initial dose.

Examination of the vertebral column and pelvic cavity revealed a total of 15 adult *P. andersoni* and *P. odocoilei* in connective tissue of the epidural and peritoneal fat in four of five deer. Worms were found only between the first lumbar and third sacral vertebrae (four worms), within an

Figure 1. Distribution of adult Parelaphostrongylus spp. in white-tailed deer and mule deer. (◖ =50% in backstraps, ● >50% in backstraps)


No. Muscle Regions
1
2
3
4
5
6
7
Days Post Exposure
50
65
80
95
110
125
30
60
90
120
150
180
210
Worms Per Deer

enlarged spinal lymph node associated with the cauda equina (one dead worm), and in connective tissue of fat lining the peritoneal cavity immediately ventral to sacral vertebrae 1 to 3 (10 worms). A few eggs and first-stage larvae were associated with these worms.

In one animal (WTD 41), 47 adult *P. andersoni* (10% of recovery) were found in connective tissue of a large fat deposit immediately overlying the lesser curvature of the abomasum.

Adults of both species were evenly distributed on the left and right sides of individual carcasses. A similar distribution was noted for females and males (where identifiable).

Orientation within the carcass

Adult worms of both species were always associated with connective tissue (Fig. 2). Over 90% of the worms recovered were coiled in deep and superficial perimysium in areas of origin, insertion, and the belly of a wide variety of muscles. Occasionally, worms were in epimysium, most commonly in the abdominal wall between the internal and external oblique muscles. Additional worms were found in connective tissue within major fat deposits between muscles. The latter two locations contributed 0-17% ($\bar{x}$=4) and 0-11% ($\bar{x}$=3), respectively, of the worms recovered from 17 individual fawns. In deer killed early in the infection (46 and 54 dPE), a few worms were associated with nervous tissue within the muscle bundles.

Figure 2. Adult female Parelaphostrongylus odocoilei in intermuscular connective tissue in the abdominal wall.

Figure 3. Adult female Parelaphostrongylus andersoni within a vein in the longissimus dorsi.

2

Adult *P. andersoni* in WTD were usually coiled and oriented parallel to the plane of fibres of adjacent muscle bundles. In contrast, adult *P. andersoni* and *P. odocoilei* in MD often did not lie flat but were interwoven between the muscle fibres. Rarely did worms overlap one another.

Females of both species were closely associated with connective tissue of the venous system. Nineteen±14 and 16±4% of female *P. andersoni* and *P. odocoilei*, respectively, were found entirely or with the posterior 1/3-2/3 within a vein or venule (Fig. 3). Males of both species were usually coiled in connective tissue adjacent to at least one female.

Associations within the carcass

Distribution of sexes within the carcass of 14 deer was examined. Overall sex ratio of *P. andersoni* and *P. odocoilei* varied little and was approximately 3 females:2 males. This ratio appeared unrelated to time after exposure, parasite species, or host species involved in the infection. Sex ratio was also similar in most individual muscles.

Worms were found as individual males, individual females, mixed pairs, mixed groups, or groups of females. Grouping of males and females into these associations was similar in *P. andersoni* and *P. odocoilei* infections (Table 3).

In comparison within each sex, the highest proportion of males was in mixed pairs ($F=30.8$, d.f.=2,39). In contrast, the percentage of females found in mixed pairs or as single worms was similar ($F=0.36$, d.f.=2,39).

TABLE 3. Groupings of males and females of Parelaphostrongylus spp. within white-tailed deer (WTD) and mule deer (MD)

	P. andersoni		P. odocoilei
	WTD	MD	MD
No. deer examined	5	4	5
Total ♂/Total ♀	283/441	135/226	231/308
Males			
as individuals	13 ± 12*	14 ± 20	19 ± 16
in mixed pairs	67 ± 23	58 ± 6	56 ± 23
in mixed groups	20 ± 17	30 ± 16	25 ± 8
in male groups	0	0	0
Females			
as individuals	39 ± 19	38 ± 15	40 ± 18
in mixed pairs	40 ± 15	34 ± 14	33 ± 17
in mixed groups	15 ± 13	23 ± 11	25 ± 19
in female groups	3 ± 7	4 ± 4	4 ± 6

*Mean percent of worms recovered per individual deer ± S.D.

First-stage larvae

First-stage larvae (L1's) of *P. andersoni* and *P. odocoilei* were found in the lymph nodes, lungs, gastro-intestinal tract, and faecal samples. L1's were usually in afferent lymphatic vessels and subcapsular spaces of the deep and superficial inguinal, bronchial, and mediastinal lymph nodes.

In the lungs, first-stage larvae were found in capillaries, alveolar spaces, and bronchial passages throughout all lobes. Larvae were quantified as number of L1's/gram wet weight lung and total number of L1's/lung. Recovery per gram of lung tissue was variable (Table 4). The number of L1's in lungs was not related to number of female worms recovered, number of larvae in faeces at death (see below), or time after exposure.

First-stage larvae were recovered from faecal samples of all fawns exposed to *P. andersoni* and *P. odocoilei*. Fawns became patent within 41-74 dPE (Table 4). No significant differences were noted between prepatent periods in different hosts or parasites. However, prepatent periods in WTD exposed to *P. odocoilei* in 1978 were significantly longer than in 1979 ($t=3.30$, d.f.=5).

Number of first-stage larvae in faeces showed two distinct patterns. The pattern of larval output in *P. andersoni* infections was consistent in WTD and MD exposed to 300 or 1000 L3's. It consisted of an exponential rise in the first 2-3 weeks of patency and peaked at approximately 4-6

TABLE 4. First-stage larvae of Parelaphostrongylus spp. in experimentally-infected white-tailed deer (WTD) and mule deer (MD).

	Prepatent period	No. adult females recovered	Larvae/g lung	Total larvae/ lung	Larvae/g faeces at necropsy	Maximum mean weekly larval output
P. andersoni						
WTD 33*	53	-	183	-	1827	1325
WTD 63	54	1	0	0	0	95
WTD 38	55	9	609	4.6×10^5	194	101
WTD 42	54	29	7882	3.0×10^6	1483	1256
WTD 39	59	31	2862	1.1×10^6	520	1032
WTD 51	51	118	450	1.5×10^5	94	996
WTD 41	51	273	1000	1.5×10^6	10120	9187
MD 39	54	36	1386	8.8×10^5	56	656
MD 27**	-	40	0	0	0	0
MD 38	49	73	599	8.0×10^5	151	617
MD 25	51	75	7323	4.1×10^6	1805	6616
P. odocoilei						
WTD 28	74	0	0	0	0	0.3
WTD 29	60	0	0	0	0	0.8
WTD 37	41	0	0	0	0	1.3
WTD 44	46	0	0	0	0	0.2
WTD 45	48	0	0	0	0	0.8
WTD 46	41	0	0	0	0	1.8
WTD 50	41	0	0	0	0.1	1.1
MD 21	50	-	2195	1.9×10^6	10605	13777
MD 41	47	65	1183	1.2×10^6	1792	3774
MD 20	50	66	558	6.4×10^5	4475	18752
MD 14	50	69	780	-	28500	25009
MD 55	48	96	7366	7.9×10^6	762	207
MD 26	45	102	19692	2.5×10^7	33	14205

* Partial results for this deer given in Pybus and Samuel (1981).
** Animal killed prior to patency.

wks postpatency (PP) (Fig. 4). The pattern of larval output in MD exposed to *Parelaphostrongylus odocoilei* (Fig. 5) was similar to that of fawns exposed to *P. andersoni* (Fig. 4). WTD exposed to *P. odocoilei* exhibited a pattern of larval output different from all other fawns exposed to worms (Fig. 5). In these WTD, output of larvae remained low-level, erratic, and short-term as daily output often dropped to zero.

Quantitatively, the number of larvae shed differed among fawns exposed to *P. andersoni*. In six deer (four WTD, two MD), mean peak larval output was 980±295 l/g (Fig. 4). Of the remaining four fawns, larval output in the two WTD was markedly low (peak output 101 and 95 l/g) while that in the one MD and one WTD was excessively high (peak output 6616 and 9187 l/g, respectively). Mean weekly larval output during the exponential phase correlated with the number of female worms recovered (for example, in wk 1, $F=33.7$, $d.f.=1,7$; $p=0.001$).

Larval output in four MD exposed to *P. odocoilei* was extremely high and peaked at 17,254 l/g in wk 6 PP (Fig. 5). In one MD, larval output peaked at 3,774 l/g in wk 10 PP. Larval output did not correlate with the number of females recovered. Mean peak output in five WTD exposed to *P. odocoilei* was 0.4 l/g in wk 4 PP. Output was similar regardless of initial dose.

In 1979, three control animals (1MD, 2WTD) passed dorsal-spined larvae in faeces (Fig. 4). The pattern of

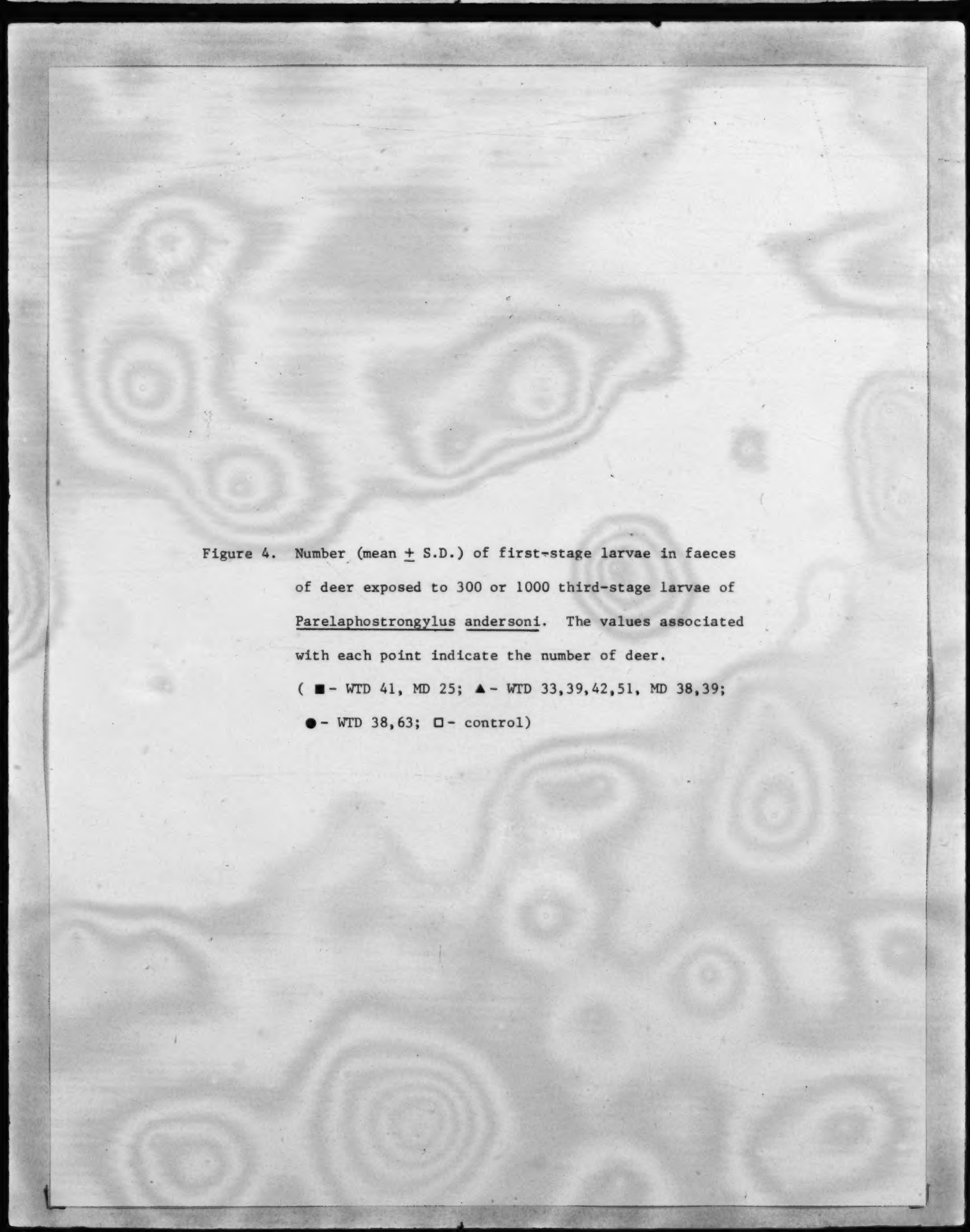
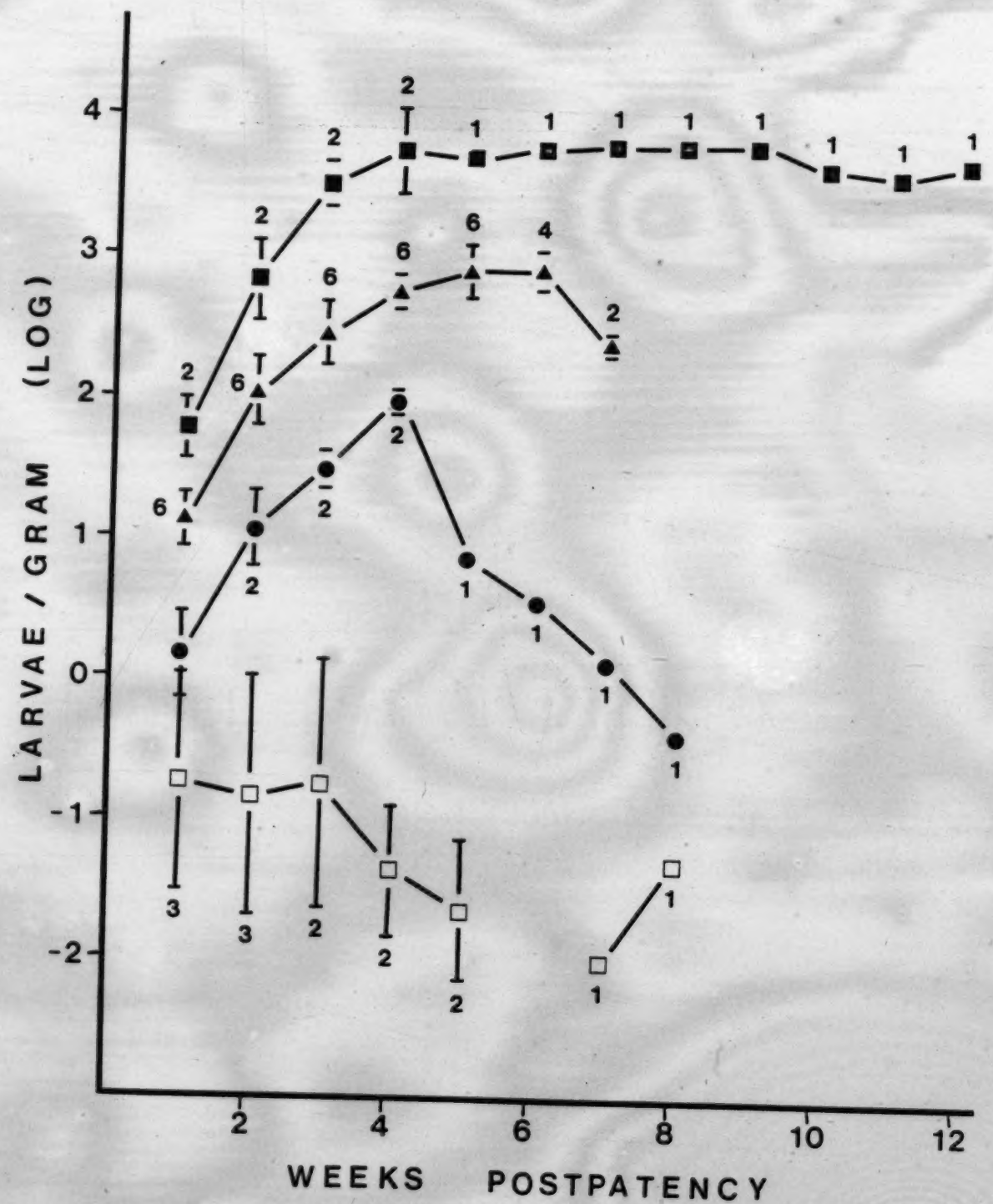


Figure 4. Number (mean ± S.D.) of first-stage larvae in faeces of deer exposed to 300 or 1000 third-stage larvae of Parelaphostrongylus andersoni. The values associated with each point indicate the number of deer.
(■ - WTD 41, MD 25; ▲ - WTD 33,39,42,51, MD 38,39;
● - WTD 38,63; □ - control)


LARVAE / GRAM (LOG)
4
3
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6
8
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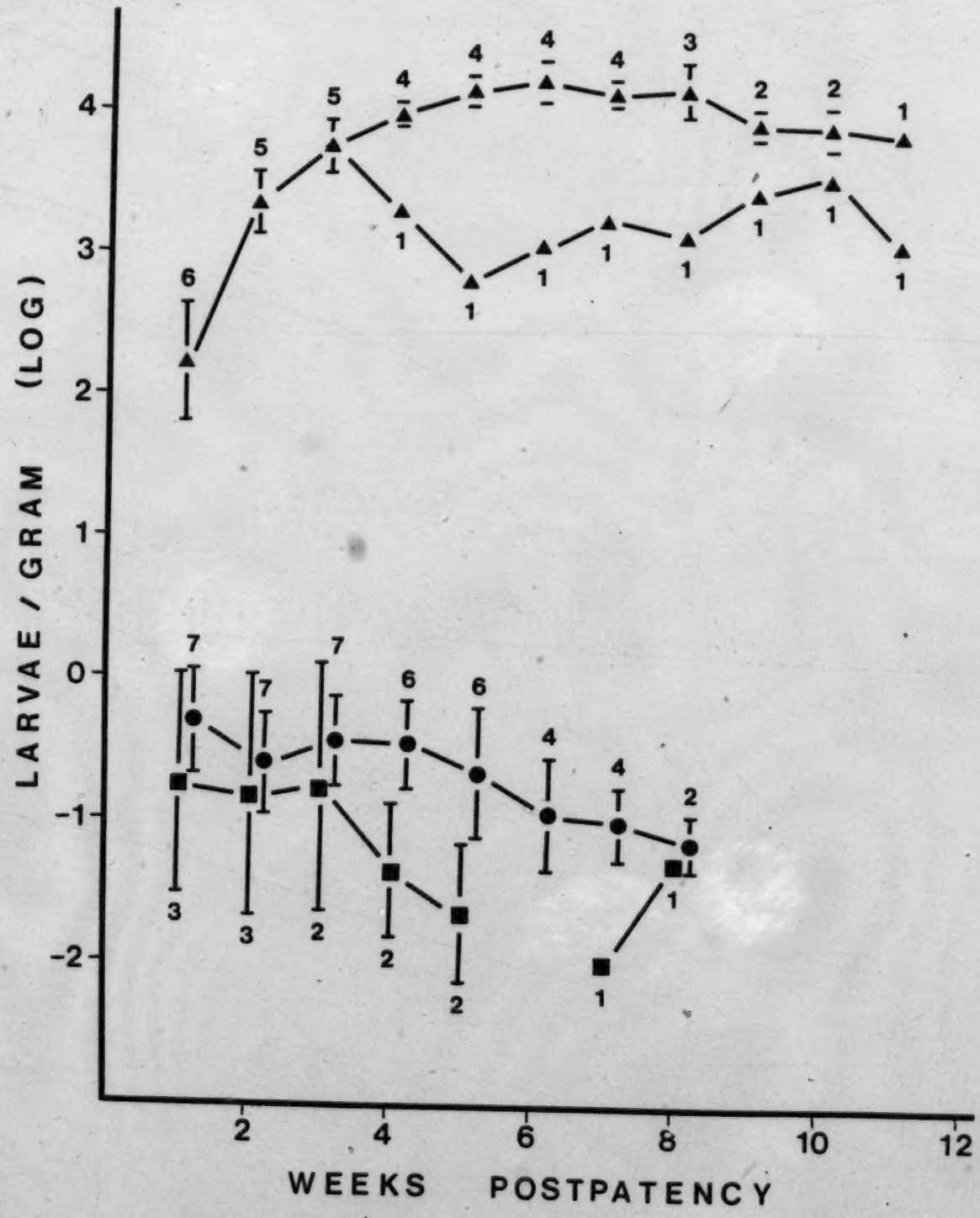
12
WEEKS POSTPATENCY

Figure 5. Number (mean ± S.D.) of first-stage larvae in faeces of deer exposed to 300 or 1000 third-stage larvae of Parelaphostrongylus odocoilei. The values associated with each point indicate the number of deer.
(▲- MD, ●- WTD, ■- control)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WEEKS POSTPATENCY

output was erratic, low-level, and short-term. Quantitatively, the number of larvae from controls was similar to that of WTD exposed to *P. odocoilei* during the first three weeks of patency. After this time, output in controls was significantly less than in experimentally-infected fawns ($t=3.26$ and 2.64, $d.f.=6$, week 4 and 5, respectively).

No adults or larvae of *Parelaphostrongylus* spp. were recovered from control animals at necropsy.

DISCUSSION

The relationship between a host and a parasite may be evaluated using a variety of criteria (see Odening 1976). Of primary importance in this relationship is the level of infectivity of the parasite, its location within the final host, and its subsequent productivity. A comparison of *P. andersoni* and *P. odocoilei* in WTD and MD indicated differences in the host-parasite relationships.

Parelaphostrongylus andersoni is capable of establishing moderate levels of infection in both WTD and MD. However, recovery rates from individual WTD were more variable than those from MD. Previous results from WTD exposed to moderate levels of *P. andersoni* also suggest some variation in individual response to infection (see Nettles and Prestwood 1976). Such variability has been described for numerous nematode infections and may relate to a variety of factors (for example, age, nutritional condition,

reproductive condition or genetic variability)(see reviews in Kennedy 1975, Holmes in press). In our study, age and condition of the fawns are similar. However, genetic variability could not be controlled.

Recovery rates in *P. odocoilei* infections were consistently high in MD but zero in WTD. Rates were higher in MD exposed to *P. odocoilei* than in those exposed to *P. andersoni*. Results suggest both worms were relatively efficient at establishing infection in MD. In contrast, the recovery rates in WTD exposed to each worm were markedly different. Reasons for such differences are currently unknown.

Parelaphostrongylus andersoni has been reported previously only from WTD (Prestwood 1972, Prestwood et al. 1974, Nettles and Prestwood 1976, Prestwood and Nettles 1977, Pursglove 1977, Pybus and Samuel 1981) and thus information concerning variation in recovery rates between different host species is lacking. However, a previous report of *P. odocoilei* in moose (Pybus and Samuel 1980) supports current observations of interspecific variation in recovery rates.

Recovery rates of adult worms in the present study were considerably higher than indicated in previous studies with this group of worms (cf. Nettles and Prestwood (1976), Prestwood and Nettles (1977) for *P. andersoni*; Platt and Samuel (1978b) for *P. odocoilei*; Anderson (1965), Anderson et al. (1966), Anderson and Strelive (1966, 1968), Nettles

et al. (1977) for *P. tenuis*; and Lankester (1977) for *E. cervi*). Mitskevich (1958) reports a moderate recovery (30%) of adult *E. cervi* from one experimentally-infected reindeer (*Rangifer tarandus*). Differences probably reflect variation in methodology. It is, however, apparent that *P. andersoni* and *P. odocoilei* can be more successful in WTD and MD, respectively, than previously suspected. These results may have important implications in understanding aspects of transmission to the final host.

Parelaphostrongylus andersoni and *P. odocoilei* were both recovered from the connective tissue in a variety of skeletal muscles. Both species were interwoven between muscle fibers in MD and the potential exists for more damage in this host (see Chapter V).

Distribution of adult *P. andersoni* and *P. odocoilei* throughout the skeletal muscles was similar and related to the duration of infection and the number of worms present but not to the dosage level (see also Appendix IV). It is unknown whether the worms in backstrap muscles actually moved to other muscles or whether those appearing in distal sites were inconspicuous until they entered the muscles. The absence of dead or degenerate adults suggests worms do not die and disappear from later infections, and lesions within muscle bundles indicated worms move through the tissues (Chapters IV and V).

Adult worms were recovered from the backstraps regardless of the length of infection or the number of worms

recovered (see also Appendix IV). These observations support the conclusion of Prestwood et al. (1974) that examination of the longissimus dorsi can be used in surveys for *P. andersoni* infections. The presence of "20 or more lesions each containing at least one *P. andersoni*" in the loins of naturally-infected deer (Anderson and Prestwood 1981, p. 302) is also similar to that seen in some of the animals in our study.

It is not known why *P. andersoni* and *P. odocoilei* move. Immature adults were recovered only from an animal killed prior to patency (46 dPE). At this time, worms were recovered from the backstraps and a few from the thighs. This suggests that sexual maturation occurs soon after the worms enter muscle tissue. Gravid females and clumps of eggs found within the spinal epidural space indicate maturation may also occur within the vertebral canal. Thus, movement through skeletal muscles does not appear related to maturation of the worms.

There is little evidence to suggest that worms move through skeletal muscle as a requirement for successful reproduction. Both species were efficient at locating the opposite sex. Males and females were paired early in the infection and associations did not change over time. Males were usually associated with one or more females throughout the infection regardless of muscle site. In addition, a high proportion of females was always associated with blood vessels. Thus, the parasites are well adapted to ensuring

fertilization of female worms and transportation of the eggs to the lungs.

However, the relationship can apparently break down. Accidental transfer of females in the blood stream could explain the observations of *P. odocoilei* in the heart of black-tailed deer (see Hobmaier and Hobmaier 1934, Brunetti 1969). Similarly, the low number of larvae in lymph nodes and their presence only in peripheral regions of the nodes indicates the lymphatics are probably not a primary route for larvae to reach the lungs.

It is possible that worms move to escape a host immune response or their own metabolic wastes (see Chapters IV and V).

Wide distribution of *P. odocoilei* within the carcass of naturally-infected black-tailed deer has been reported (Hobmaier and Hobmaier 1934, Brunetti 1969). These authors also noted a predilection of pairs of worms (1male/1female) for connective tissue associated with lymphatics and blood vessels and Brunetti reported eggs and larvae in lymph nodes. A similar distribution of *P. odocoilei* in experimentally-infected moose also has been described (Pybus and Samuel 1980).

P. andersoni is previously reported only from skeletal muscles of the loin and thighs of WTD (Prestwood 1972, Prestwood et al. 1974, Nettles and Prestwood 1976, Prestwood and Nettles 1977, Pybus and Samuel 1981). The apparent localization of worms to these muscles appears to be a

function of necropsy methods. More extensive search of the carcass (a goal in our study) revealed worms throughout most skeletal muscles after 100 dPE, particularly in deer harboring more than 100 adult worms.

Parelaphostrongylus andersoni and *P. odocoilei* have not been reported previously from the vertebral canal. Within the canal, worms were recovered only from epidural tissues and spaces. Note that standard methods for examination of the spinal cord for *P. tenuis* and *E. cervi* failed to reveal *P. andersoni* and *P. odocoilei*. Only when the method changed to examination of the vertebral canal rather than the spinal cord, were worms recovered. Worms could easily have been missed in the vertebral canal of animals examined early in this study. It is possible that failure to recover adult worms from WTD exposed to *P. odocoilei* may be associated with improper examination of the vertebral canal, however, this awaits further investigation.

The early stages of migration of *P. andersoni* and *P. odocoilei* within the definitive host may be similar to that suggested for *P. tenuis* by Anderson (1963). He concluded that third-stage larvae of *P. tenuis* leave the gut through the abomasal wall, and move to the lumbar region where they develop to fifth-stage within the CNS. They then migrate anteriorly towards the brain. A similar development has been suggested for *E. cervi* (see Anderson, 1968, Lankester 1977). It is worth speculation that the two muscleworm species may also move to the lumbar region early in their migration.

Subsequently, they appear to disperse from this region into adjacent muscles.

The pattern of larval output in the faeces of three control animals in 1979 and the failure to recover adult worms casts doubt on the data from WTD exposed to *P. odocoilei*. In addition, Platt and Samuel (1978a) were unable to establish *P. odocoilei* infections in eight WTD fawns and concluded WTD were a refractory host for this worm.

However, the pattern of output was similar in all WTD exposed to *P. odocoilei* during this study. The initial output in WTD exposed in 1979 may have been from the same unidentified infection detected in controls. Larval output in 1979 WTD rose slightly and became significantly different from that of controls at 3-4 weeks postpatency (62-72 dPE). This resembled the prepatent period seen in 1978 (60, 74 d) and indicated larvae passed after this time may have included those of *P. odocoilei*. Contamination of samples in the laboratory is possible, but considered unlikely. However, without confirmation by recovery and identification of adult worms, it is not possible to determine whether *P. odocoilei* established an infection in WTD. It is known that deer in this study were exposed to *P. odocoilei* and subsequently passed larvae in faeces in a similar pattern.

The pattern and timing of larval output in MD exposed to *P. odocoilei* are similar to previous reports (Platt and Samuel 1978a). Comparable data for *P. andersoni* are not available. In a direct comparison of productivity (as

reflected by larval output), larval output reached similar moderate levels in WTD and MD exposed to *P. andersoni*. In contrast, productivity was markedly higher in MD than in WTD exposed to *P. odocoilei*. These differences appear directly related to differences in infectivity (or per cent recovery) in different host-parasite combinations.

MD were not previously known as suitable hosts for *P. andersoni*. Worm establishment and larval output similar to that in WTD indicated MD could act as a primary host in maintaining populations of *P. andersoni*. A recent report of *P. andersoni* from southeastern British Columbia (Pybus and Samuel 1981) indicates the worm is present within the range of MD. Investigators in western Canada and the United States should be aware of the potential for infection of MD with either or both *P. andersoni* or *P. odocoilei*.

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LITERATURE CITED

Anderson, R. C. 1963. The incidence, development, and experimental transmission of *Pneumostrongylus tenuis* Dougherty (Metastrongyloidea: Protostrongylidae) of the meninges of the white-tailed deer (*Odocoileus virginianus borealis*) in Ontario. Can. J. Zool. **41**: 775-792.

----- 1965. The development of *Pneumostrongylus tenuis* in the central nervous system of white-tailed deer. Path. Vet. **2**: 360-379.

----- 1968. The pathogenesis and transmission of neurotropic and accidental nematode parasites of the central nervous system of mammals and birds. Helminthol. Abstr. **37**: 191-210.

----- 1971. Lungworms. *In*: Parasitic diseases of wild mammals. J.W. Davis and R.C. Anderson (eds.). Iowa State Univ. Press, Ames, Iowa. pp. 81-126.

Anderson, R.C. and A.K. Prestwood. 1981. Lungworms. *In*: Diseases and parasites of white-tailed deer. W.R. Davidson (ed.), Tall Timbers Research Station Miscell. Publ. No. 7., Tallahassee. pp 266-317.

Anderson, R.C., M.W. Lankester, and U.R. Strelive. 1966. Further experimental studies of *Pneumostrongylus tenuis* in cervids. Can. J. Zool. **44**: 851-861.

Anderson, R.C., and U.R. Strelive. 1966. Experimental cerebrospinal nematodiasis (*Pneumostrongylus tenuis*) in sheep. Can. J. Zool. **44**: 889-894.

-----. 1968. The experimental transmission of *Pneumostrongylus tenuis* to caribou (*Rangifer tarandus terraenovae*). Can. J. Zool. **46**: 503-510.

Brunetti, O.A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game **55**: 307-316.

Chitwood, B.G., and M.B. Chitwood. 1950. Introduction to

Nematology. University Park Press, Baltimore, London, Tokyo. 334pp.

Hobmaier, A., and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n. sp., a new lungworm in black tail deer (*Odocoileus columbianus*). Description and life history. Proc. Soc. Exp. Biol. Med. **31**: 509-514.

Holmes, J.C. in press. Evolutionary relationships between parasitic helminths and their hosts. *In*: Coevolution. D.J. Futuyma, M. Slatkin, J. Roughgarden, and B. Levin (eds.). Sinauer Associates, Sunderland, Mass. 82pp.

Kennedy, C.R. 1975. Ecological animal parasitology. Blackwell Scientific Pub., Oxford. 163pp.

Lankester, M.W. 1977. Neurologic disease in moose caused by *Elaphostrongylus cervi* Cameron 1931 from caribou. Proc. N. Am. Moose Conf. and Workshop. **13**: 177-190.

Lankester, M.W., and R.C. Anderson. 1971. The route of migration and pathogenesis of *Skrjabingylus* spp. (Nematoda: Metastrongyloidea) in mustelids. Can. J. Zool. **49**: 1283-1293.

Mackerras, M.J., and D.F. Sandars. 1955. The life history of the rat lungworm, *Angiostrongylus cantonensis* (Chen)(Nematoda: Metastrongylidae). Aust. J. Zool. **3**: 1-21.

Mitskevitch V.Y. 1958. The elucidation of the life cycle of the nematode *Elaphostrongylus rangiferi* sp. nov. from the reindeer. AKad. Nauk. USSR. **119**: 253-255.

Nettles, V.F., and A. K. Prestwood. 1976. Experimental *Parelaphostrongylus andersoni* infections in white-tailed deer. Vet. Pathol. **13**: 381-393.

Nettles, V.F., A.K. Prestwood, R.G. Nichols, and C.J. Whitehead. 1977. Meningeal worm-induced neurologic disease in black-tailed deer. J. Wildl. Dis. **13**: 137-143.

Odening, K. 1976. Conception and terminology of hosts in parasitology. Adv. in Parasitol. 14: 1-93.

Platt, T.R., and W.M. Samuel. 1978a. *Parelaphostrongylus odocoilei*: life cycle in experimentally infected cervids including the mule deer, *Odocoileus h. hemionus*. Exp. Parasitol. **46**: 330-338.

----- 1978b. A redescription and neotype designation for *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea). J. Parasitol. **64**: 226-232.

Prestwood, A.K. 1972. *Parelaphostrongylus andersoni* sp. n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus virginianus*). J. Parasitol. **58**: 897-902.

Prestwood, A.K., and V.F. Nettles. 1977. Repeated low-level infection of white-tailed deer with *Parelaphostrongylus andersoni*. J. Parasitol. **63**: 974-978.

Prestwood, A.K., V.F. Nettles, and F.E. Kellogg. 1974. Distribution of muscleworm, *Parelaphostrongylus andersoni*, among white-tailed deer of the southeastern United States. J. Wildl. Dis. **10**: 404-409.

Pursglove, S.R. 1977. Helminth parasites of white-tailed deer (*Odocoileus virginianus*) from New Jersey and Oklahoma. Proc. Helminth. Soc. Wash. **44**: 107-108.

Pybus, M.J., and W.M. Samuel. 1980. Pathology of the muscleworm, *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea), in moose. Proc. N. Am. Moose Conf. and Workshop **16**: 152-170.

----- 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. **45**: 537-542.

IV. Pathology of *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) in two cervid hosts.

ABSTRACT

Seven white-tailed deer and four mule deer were each exposed to 300 or 1000 third-stage larvae of *Parelaphostrongylus andersoni*. Gross and histopathologic lesions are described from a variety of tissues. Major gross lesions were focal haemorrhage throughout lungs and skeletal muscles. Large mononuclear cells and, occasionally, eosinophils accumulated around eggs and larvae in muscles, lungs, and lymph nodes. There was general activation of the lymphoid system. Lesions were characterized as focal myositis, moderate verminous pneumonia, and mild to moderate lymphadenitis. Quantitative evaluation of pulmonary lesions indicated progressive tissue damage throughout infection.

Response to infection with *Parelaphostrongylus andersoni* was generally similar in white-tailed deer and mule deer; however, severity of damage appeared slightly greater in mule deer. Variation in response indicated differing individual susceptibility and suggested that host response may play a major role in determining effects of *P. andersoni* in the final host. Response was characterized by the induction of both humoral and cell-mediated immunity.

INTRODUCTION

Parelaphostrongylus andersoni Prestwood 1972, a metastrongyloid nematode, was described from the skeletal muscles of white-tailed deer (*Odocoileus virginianus*) (WTD)(Prestwood 1972). Further investigation of this worm has been restricted largely to its geographic distribution (Prestwood et el. 1974, Pursglove 1977, Pybus and Samuel 1981).

The life cycle of *P. andersoni* within the final host is apparently similar to that of closely-related metastrongyles. Within this group, infective larvae penetrate the abomasal wall (Anderson and Strelive 1967, Samuel unpub.) and migrate by an unknown route to the skeletal muscles (Chapter III) and/or vertebral canal (Anderson and Strelive 1967) of the lumbar region. During infection, adult worms move within the epimysium and perimysium of skeletal muscles throughout the definitive host (Chapter III). Female worms apparently penetrate intramuscular veins and venules and release eggs directly into the circulatory system. Occasionally, eggs are released into muscle tissue and some eggs and larvae are filtered out in regional lymph nodes (Chapter III). However, many eggs are carried to the capillaries of the lungs where they are filtered from the blood. After the eggs hatch, larvae move up the airways of the respiratory system, are subsequently swallowed, and leave the host with the feces.

Clinical and covert disease have been investigated in WTD experimentally infected with *P. andersoni* (Nettles and Prestwood 1976). Major lesions were eosinophilic myositis in the back and thighs and granulomatous accumulations of mononuclear cells around eggs and larvae in the lung. This paper reports additional information of the pathology of *P. andersoni* in WTD.

Parelaphostrongylus andersoni has been reported previously only from WTD. A recent report of this worm in southeastern British Columbia (Pybus and Samuel 1981) indicates the parasite is present in populations of WTD sympatric with mule deer (*Odocoileus hemionus hemionus*)(MD). Thus, the potential exists for MD to be exposed to this worm. This paper therefore also evaluates the pathology of *P. andersoni* in experimentally-infected MD.

MATERIALS AND METHODS

Eleven neonatal WTD and 6 MD were collected throughout Alberta in 1978-80. Animals were transported to the University of Alberta Biomedical Animal Centre, Ellerslie, Alberta and maintained as outlined (Chapter II). In 1978 an initial source of *P. andersoni* was obtained from naturally-infected WTD in southeastern British Columbia (Pybus and Samuel 1981). All subsequent infections involved larvae derived from 1978 infections and methods as described (Pybus and Samuel 1981).

After weaning, 11 fawns approximately 12 weeks old were each exposed per os to 300 or 1000 third-stage larvae of *P. andersoni* (one animal received 388 larvae)(Table I). Six control animals were treated similarly excluding exposure to worms. Throughout the experiments, fawns were observed for clinical evidence of disease. Blood samples were taken weekly from WTD starting at least 2 weeks prior to exposure to worms and continuing until the animal was killed. Ten cc of blood were taken from a jugular vein with a 21-gauge infusion set during manual restraint. All samples were collected within 5 minutes after the two researchers entered the pen. Total RBC, total WBC, haematocrit, and haemoglobin were evaluated using a Coulter Counter Model B (for total cell counts), haematocrit centrifuge, and haemoglobinometer, respectively. Differential WBC counts were determined from thin blood smears.

For analysis of haematologic values, ANOVA on data grouped by treatment (infected and control) in each of 3 time periods (pre-exposure, pre-patent, and post-patent) was followed by pairwise comparison of group means. Time periods for control animals were arbitrarily chosen to correspond to the mean of those of infected animals. WTD 41 was deleted from analysis of eosinophil levels (see Discussion).

Mean weekly weight gains during pre-exposure, pre-patent, and post-patent periods, as well as throughout the entire infection, were calculated. Mean values for control and experimental animals were compared by an

Table I. Experimental design to investigate the pathology of *Parelaphostrongylus andersoni* in fawns.

	White-tailed deer				Mule deer				Control
	Deer number	Dose	dPE*	Worm recovery**	Deer number	Dose	dPE	Worm recovery	Deer number
1978	WTD 33	388	98	30	-				WTD 26
									WTD 30
1979	WTD 38	300	86	4	MD 25	300	138	45	WTD 40
	WTD 39	300	100	20	MD 27	300	46	19	WTD 43
	WTD 41	1000	79	47					MD 30
	WTD 42	300	90	18					
	WTD 51	1000	104	21					
1980	WTD 63	300	108	1	MD 38	300	82	39	MD 36
					MD 39	300	104	21	

* Days post exposure when animal killed.
** Expressed as percentage of dose.

analysis of covariance.

Animals were killed at various times after exposure by intravenous injection of sodium pentabarbitol (Table I). Body condition at necropsy was assessed by a subjective evaluation of the amount and distribution of subcutaneous and visceral fat using criteria similar to Stockle et al. (1978). General muscle condition was also assessed. Categories were as follows:

1) poor - Fat deposits lacking. Muscles flaccid and atrophic.

2) fair - Some visceral fat present. Muscles fleshed out.

3) good - Visceral fat deposits extensive. Some subcutaneous fat present but deposits small.

4) very good - Extensive visceral and subcutaneous deposits. Perivascular fat present.

5) excellent - As in previous category plus presence of intramuscular fat deposits.

At necropsy, the carcass was searched for gross evidence of infection. Particular attention was given to skeletal muscles, central nervous system, and vertebral canal. Tissues for histologic study were taken within 1/2-1 hour after death and fixed in 10% buffered formalin. Samples were embedded in paraffin, sectioned at 7um and stained with haematoxylin and eosin.

Gross lung lesions were assessed using the following categories:

1) normal - No discolouration. Lungs pink and soft.

2) minor - Slight discolouration. Few small haemorrhages present. Minor increase in size.

3) moderate - Petechial haemorrhage throughout all lobes.

4) heavy - Grey discolouration in some lobes. Haemorrhage throughout. Some firmness.

5) severe - Yellow discolouration throughout. Red-purple discolouration present in some lobes. Firm throughout. Greatly enlarged in all lobes.

Assessment of the extent of damage to lung tissue was based on tissue samples taken from two specific regions in the diaphragmatic and cardiac lobes of each lung. Diameter of up to 50 granulomas within each lung section was measured using an ocular micrometer at 100X magnification (see Lichtenberg and Mekbel 1962). Remaining granulomas were counted. An outline of the area examined was drawn with the aid of a drawing tube and measured with a planimeter.

Similarly, areas of consolidated tissue in each section were mapped and measured. Data were expressed as percentage of tissue involved in consolidation in each section examined (% consolidation).

Quantitative data were first analyzed for differences between left and right side, and diaphragmatic and cardiac lobes. The four values were combined for each deer and then analyzed by ANOVA. Variances between groups were disparate and non-homogeneous. Log transformation reduced the heteroscadasticity in the data and, thus, statistical analyses were performed on transformed data.

The number of cells, type of cells, and number and size of germinal centers present in lymph nodes were assessed (following suggestions of Cottier et al. 1972). Four categories of activity were used:

1) inactive - No delineation of germinal centers from surrounding cortex. Cortical space open and well defined. Very few histiocytes. Eosinophils and neutrophils absent. Medullary sinuses relatively acellular. Medullary cords small.

2) minor - A few delineated germinal centers in cortex. Few cells in medullary sinuses.

3) moderate - Some centers activated. Paracortical region very cellular and extending into proximal medulla.

4) heavy - Many active centers. Centers cuffed with lymphocytes. Extensive and cellular paracortical region. Medulla reduced. Medullary sinuses cellular.

The modal activity value for each node was determined for animals grouped as controls, WTD, and MD. In addition, pathology in individual nodes was compared by activity and number of deer in which a node was affected.

Activity of splenic lymphoid tissue was classified as follows:

1) inactive - No germinal centers. Splenic cords small and few in number. Red pulp relatively acellular.

2) minor - A few germinal centers widely dispersed throughout tissue. Centers relatively inactive. Periarterial accumulations of lymphocytes moderate in size. A few white

blood cells, largely lymphocytes, present in splenic sinuses.

3) moderate - Some small active germinal centers. Wide sheaths of lymphocytes on arteries. Moderate cellularity in splenic sinuses.

4) heavy - Large, active germinal centers throughout tissue. Heavy periarterial lymphatic accumulations. Cellularity in red pulp variable but always characterized by plasma cells.

Sample sizes were too small to allow statistical analysis of dosage effects. However, data appeared similar and results were combined. Some missing values were unavoidable and sample sizes are indicated for each analysis. A significance level of $p<0.05$ was accepted for all analyses.

RESULTS

Clinical signs and haematology

No fawns showed clinical evidence of infection with *Parelaphostrongylus andersoni* prior to 20 days post exposure (dPE). One MD exhibited an abnormal gait from 21 dPE until killed. It was weak in the right hind quarter and forward movement of the right hind leg was impaired. Two MD fawns exposed to 300 third-stage larvae exhibited dyspnoea which started at 70 dPE and continued until termination of the animals. However, they did not stop feeding and weight gain remained normal during this time.

Similarly, one fawn (WTD 41) exposed to 1000 third-stage larvae exhibited dyspnoea during days 75 to 79 post exposure and gasped after minimal exercise. The animal ceased feeding and lost weight before being killed at 79 dPE. At death, copious amounts of haemorrhagic foam were discharged from the mouth and nares.

No clinical signs were observed in any other animals.

Absolute and differential eosinophil values were similar in all WTD, except WTD 41, during the pre-exposure period. After exposure, these values in infected deer were significantly higher than those of controls ($F(1,132)=64.13$, $p<0.0001$) and remained high until the animals were killed. In WTD 41, no eosinophils or basophils were seen at any time in peripheral blood smears. All other blood values were similar in infected and control animals.

General condition

Body condition of seven WTD exposed to *P. andersoni* was rated very good in two animals (WTD 33,63) and good in five (WTD 38,39,41,42,51). One MD exposed to worms was rated very good (MD 39), two were good (MD 25,38), and the remaining animal, which harboured a chronic mycobacterial infection, was in poor condition (MD 27). Three control WTD were rated excellent (WTD 26,40,43), one WTD (WTD 30) and one MD (MD 30) were very good.

No differences were noted in weight gains of control or infected animals at any period during the infection.

Muscle pathology (Figs. 1-4)

Adult *P. andersoni* were recovered from skeletal muscles of all deer exposed to larvae. The characteristic gross muscle lesion in both host species was focal haemorrhage 5-15 mm in diameter. All worms recovered were either directly within a haemorrhage or, more commonly, within 10 mm of a haemorrhage that was up to 100 mm^2 in area. Not all haemorrhages were associated with worms. Pooling of thick, black blood between muscle fibres was seen occasionally in all MD. Focal necrosis was common throughout muscles of the back and thighs of deer killed after 100 dPE.

The characteristic histologic lesions were extensive cellular accumulations and tissue damage around eggs and larvae in skeletal muscles and associated fat. In WTD, macrophages were the predominent cell type with neutrophils, giant cells, eosinophils, and a few lymphocytes present in some lesions. In adjacent tissues, giant cells and plasma cells were present between pale, swollen, muscle fibres. Vasculitis and congestion were common in these areas.

Three WTD showed responses which differed from that described. One animal killed 79 dPE (WTD 41) had extensive pooling of viscous black blood throughout connective tissue and fascia of muscles of the back and hind quarters. Muscle tissue was friable. Cellular response to insult in WTD 41 was minimal relative to other infected deer. Haemorrhage and tissue degeneration were accompanied by only minor accumulations of lymphocytes. Muscle fibres were swollen and

Figure 1. Gross haemorrhage in the psoas muscle of a MD exposed to Parelaphostrongylus andersoni.

Figure 2. Myositis associated with eggs of Parelaphostrongylus andersoni deposited within the biceps femoris of a WTD. Bar = 230 um

Figure 3. Localized necrosis and diffuse tissue degeneration associated with degenerate eggs of Parelaphostrongylus andersoni in the latissimus dorsi of a WTD. Bar = 180 um

Figure 4. Venous thrombus containing eggs and inflammatory cells in the semimembranosus of a MD. The adjacent artery is unaffected. Bar = 230 um

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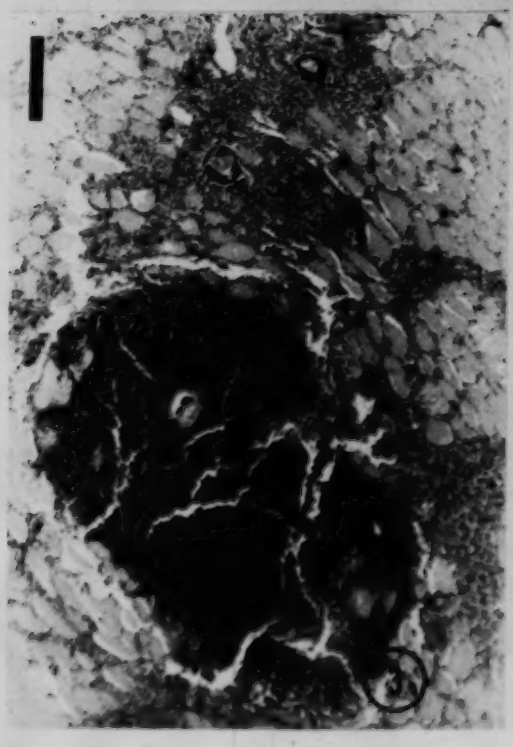
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eosinophilic. Eggs in muscle were associated with a few large mononuclear cells.

In WTD 38 and WTD 63, extensive focal necrosis (1-4mm diam) was noted throughout muscles of the backstraps and thighs. These areas contained a core of degenerate eggs embedded in a dense eosinophilic material. A few long thin areas (20X3 mm) of necrosis were seen in thigh muscles. Eosinophils were the predominant cell type associated with tissue damage while plasma cells were common throughout muscle tissue surrounding adult worms or groups of eggs.

The cellular response in MD differed slightly from that in WTD. In MD, eosinophils and/or neutrophils were the predominant cell types in damaged areas. Haemorrhage, necrosis, and muscle degeneration were common in tissues adjacent to groups of nematode eggs. Eosinophils and plasma cells were associated with diffuse muscle degeneration throughout most sections. Degranulated eosinophils were seen throughout areas of tissue damage. Vasculitis was common and vessels were cuffed with eosinophils and plasma cells. A few nerves within damaged areas contained swollen fibres with large mononuclear cells between the fibres.

In all infected deer, little or no cellular response was seen directly adjacent to adult worms in the epimysium and perimysium. Occasionally, accumulations of free erythrocytes and a few neutrophils and mononuclear cells were seen within 2 mm of a worm. Response was also minimal around female worms entirely within the lumen of a vein.

Rarely, venous thrombus was observed. Haemorrhage and inflammatory cells were present where a vessel was penetrated.

Lung pathology (Figs. 5-8)

Lungs of the two control MD and three of the four control WTD were grossly normal. One animal had a few isolated haemorrhages in one diaphragmatic lobe and was rated as minor damage. Damage to the lungs of WTD exposed to *P. andersoni* was rated moderate in six deer and heavy in one deer. Total lung weights of these deer ($\bar{x}$=490±175 g) were significantly greater than those of controls ($\bar{x}$=249±64 g)($F(1,7)=6.76$, $p<0.04$). Damage to the lungs of two MD exposed to *P. andersoni* was ranked severe; one was ranked heavy. Total lung weights of infected MD ($\bar{x}$=842±426 g) were significantly greater than those of control deer (280, 360 g) ($F(1,8)=10.21$, $p<0.02$).

Histologically, control WTD and MD showed a consistent mild chronic bronchitis and bronchiolitis with focal to diffuse atelectasis and thickening of alveolar walls. Mild to severe peribronchial and peribronchiolar lymphocytic activity was noted. Vessels were congested and cuffed occasionally with a few lymphocytes. Minor haemorrhage into adjacent parenchyma was seen around a few vessels. Hyperplasia of bronchial epithelium was noted in some sections, while respiratory bronchioles and associated arterioles occasionally were obliterated with lymphocytes.

Figure 5. Emphysema and loss of functional lung tissue due to granulomas around eggs and larvae of Parelaphostrongylus andersoni. Bar = 230 um

Figure 6. Necrosis of pulmonary granulomas in a MD. Bar = 230 um

Figure 7. Accumulation of eggs of Parelaphostrongylus andersoni within dilated pulmonary vessels. Bar = 44 um

Figure 8. Development to larvated eggs of Parelaphostrongylus andersoni within dilated blood vessels. Bar = 44 um

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Lesions were indicative of a mild inflammatory response to a common irritant in the environment, dust, for example.

Histopathologic lesions in experimental fawns were grouped into 3 categories -lesions of air passages, lesions of blood vessels, and granuloma formations. The type of lesions in the first two categories were similar to those noted in control animals and are detailed in Table II. However, only lesions more extensive than in control animals and common to a majority of experimental animals are included.

Granulomas around eggs and/or larvae were noted in the lung parenchyma of all infected animals. In 4 WTD fawns, the predominant inflammatory cells were large lymphocytes and macrophages. Lymphocytes, neutrophils, and eosinophils predominated in the remaining 3 animals. Haemorrhage and arteritis were usually associated with the granulomas.

Extensive necrosis was seen in only one WTD. In WTD 63, approximately 70% of the pulmonary granulomas consisted of a core of caseous material (including remnants of degenerate eggs and/or larvae) surrounded by giant cells and epithelioid cells embedded in proteinaceous material. Numerous eosinophils were found throughout the granulomas and adjacent tissues.

Histopathologic lesions seen in the airways and circulatory system of lungs of MD exposed to *P. andersoni* were similar to those seen in WTD (Table II). However, other histologic results indicated a more severe response in MD.

Table II. Histopathology of lungs of white-tailed deer (WTD) and mule deer (MD) exposed to *Parelaphostrongylus andersoni*.

	Air passages				Circulatory system		
	Thickened alveolar walls	Emphysema	Bronchitis and bronchiolitis	Occlusion of respiratory bronchioles	Haemorrhage	Arteritis	Lymphoid hyperplasia
WTD 33	variable	moderate	minor	minor	focal	minor	--*
WTD 38	variable	minor	severe	--	--	severe	extensive
WTD 39	variable	minor	moderate	--	--	minor	--
WTD 41	minor	minor	severe	severe	extensive	severe	--
WTD 42	moderate	minor	minor	--	focal	moderate	moderate
WTD 51	variable	moderate	--	minor	extensive	severe	--
WTD 63	moderate	minor	severe	--	extensive	severe	minor
MD 25	minor	moderate	moderate	severe	focal	moderate	extensive
MD 38	moderate	moderate	moderate	moderate	focal	severe	moderate
MD 39	moderate	moderate	moderate	moderate	lobular	severe	moderate

* Lesion not present.

Granulomas around eggs and/or larvae were more discrete in MD, with lymphocytes and macrophages the predominant cells present. Necrosis of the central region of the granuloma was present in all MD and involved an average of 38±25 per cent of the granulomas. Eosinophils and pink amorphous material were present around eggs and degenerating larvae. A few plasma cells were seen in the periphery of granulomas with necrotic centers.

Percentage of lung tissue altered by consolidation was determined in each of four lobes in each WTD. No significant variation was noted among lobes ($F(3,24)=0.27$, $p>0.05$), thus, data are combined within deer (Table III). Significant variation was noted among individual WTD exposed to *P. andersoni* ($F(6,21)=3.49$, $p<0.02$). Similarly, density and mean granuloma size also varied significantly among individuals ($F(6,21)=12.58$ and 18.28, respectively, $p<0.001$).

Density of granulomas per mm^2 of lung tissue increased with increasing number of female worms recovered ($y=0.32X10^{-2}x+0.6$, $r^2=0.50$). [Note that the low regression slope results from a small range in density but a large range in number of females recovered]. Mean granuloma size (um diameter) increased throughout infection (dPE) ($y=5.0x-281$, $r^2=0.67$). If WTD 41 and 63 (2 animals which showed an atypical response - see Discussion) are deleted from the analyses, the regression of mean granuloma size against time is strengthened ($y=5.44x-343$, $r^2=0.85$). In addition,

Table III. Quantitative histopathology of lungs of white-tailed deer (WTD) and mule deer (MD) fawns exposed to *Parelaphostrongylus andersoni* and of control animals.

	Percent consolidation	Mean granuloma size (um)	Granuloma density (No./mm^2)
WTD 33	22±4*	197±16	1.7±0.6
WTD 38	35±12	123±11	0.5±0.2
WTD 39	25±11	188±25	0.7±0.1
WTD 41	45±22	149±43	1.6±0.5
WTD 42	47±12	149±10	1.1±0.3
WTD 51	25±6	227±8	0.7±0.1
WTD 63	19±10	297±31	0.5±0.2
MD 25	17±5	178±25	1.5±0.7
MD 38	29±6	202±8	1.4±0.4
MD 39	43±21	131±9	1.6±0.5
WTD 26**	2±2	-	0
WTD 30**	6±3	-	0
WTD 40**	15±10	-	0

* Mean of four lobes ±S.D.
** Control fawn.

granuloma size was directly related to number of females recovered ($y=0.84x +132$, $r^2=0.72$) and density correlated with total number of first-stage larvae in the lungs ($y=0.23X10^6x +0.42$, $r^2=0.68$). Extent of consolidation was not correlated with any measured factor.

No significant variation in percent consolidation and density was seen among three MD exposed to *P. andersoni* (Table III). However, mean size of granulomas was significantly different between individuals ($F(2,466)=49.09$, $p<0.0001$). Sample size was too small to further investigate this difference.

No quantitative histopathological differences were noted between the two host species.

No granulomas were seen in the lungs of the three control WTD examined. Consolidation was present but was significantly less than in infected fawns ($t(50)=5.01$, $p<0.001$) (Table III).

Lymph node and spleen pathology (Figs. 9-12)

Lymph nodes were grossly enlarged, haemorrhagic, and oedematous in three of six infected WTD and all four infected MD. Gross lesions were consistently seen in deep inguinal nodes but were variable in other nodes. Lymph nodes in the remaining three WTD showed no gross lesions and were similar to those in control animals.

Control animals showed only minor histologic lesions in lymph nodes. Vascular congestion was seen in mesenteric and

Figure 9. Thrombus of eggs of Parelaphostrongylus andersoni and inflammatory cells in an afferent lymphatic of a WTD. Note the active germinal centres. Bar = 250 um

Figure 10. An afferent lymphatic in WTD 41 occluded with larvae of Parelaphostrongylus andersoni. No response in the germinal centres. Bar = 233 um

Figure 11. Granulomas associated with eggs of Parelaphostrongylus andersoni in the subcapsular sinus in a lymph node of a MD. Bar = 250 um

Figure 12. Granulomas associated with eggs of Parelaphostrongylus andersoni in the paracortical region in a lymph node of a MD. Bar = 250 um

9

10

ruminal nodes of one animal and ruminal node of another. Haemorrhage, haemosiderin, oedema, and free neutrophils in the medulla were present in some controls. Only lesions markedly different from those in control animals are described in infected fawns.

Histologic lesions in lymph nodes of infected deer were variable. The primary lesion was extensive haemorrhage throughout the medulla. Histiocytes, neutrophils, and a few eosinophils were found within haemorrhagic areas and some degeneration of lymphocytes in medullary cords was noted. Haemorrhage was common in deep inguinal (five WTD, two MD), axillary (three WTD, two MD), and bronchial (two WTD, one MD) lymph nodes. Mesenteric lymph nodes consistently contained siderocytes and free haemosiderin throughout the medulla.

Granulomas were observed around eggs and larvae of *P. andersoni* in afferent lymphatics, subcapsular sinuses, and, rarely, in trabeculae at the corticomedullary junction in a variety of lymph nodes (Table IV). Afferent lymphatic vessels were dilated and occluded with well-defined thrombi. These thrombi consisted of eggs and larvae embedded in pink-staining amorphous material with giant cells and a few small lymphocytes nearby. Occasionally, degenerate eggs and larvae in the center of the thrombus were surrounded by eosinophils and a few lymphocytes in layers of loose connective tissue. Increased cellularity (largely lymphocytes and eosinophils) was noted in the capsule

Table IV. Histopathology of lymph nodes and spleen of control animals and fawns exposed to *Parelaphostrongylus andersoni*.

	Activity level			Number of nodes containing granulomas		
	Control*	White-tailed deer	Mule deer	Control	White-tailed deer	Mule deer
N	4	7	3	4	7	3
Axillary	inactive**	moderate	variable	0	0	0
Bronchial	-	heavy	moderate	-	1	2
Inguinal	minor	heavy	heavy	0	5	2
Mediastinal	-	minor	moderate	-	2	3
Mesenteric	moderate	heavy	minor	0	0	0
Ruminal	heavy	heavy	variable	0	0	1
OVERALL	inactive	heavy	heavy	-	-	-
Spleen	inactive	minor	moderate	-	-	-

* White-tailed deer only.
** Modal values. See text for details.

overlying thrombosed lymphatic vessls. Cortical sinuses were occluded and germinal centers hyperplastic. Response appeared to be more common in MD than WTD.

One animal (WTD 41) exhibited lesions different from other animals. In the superficial inguinal nodes, no cellular response was associated with nematode larvae in afferent lymphatics, subcapsular sinuses, or connective tissue overlying the capsule. Germinal centers were inactive. In the deep inguinal, bronchial, and mediastinal lymph nodes, larvae in afferent lymphatics were associated with a minor granulomatous inflammation. Larvae were distributed throughout the cortex and were extended rather than coiled.

Lymphoid hyperplasia was noted in infected fawns. These data for MD must be treated cautiously as controls were not available.

Individual lymph nodes were assessed by cellular activity and frequency of pathologic involvement. Cellular responses were variable and overlapped control values (Table IV) so that no clear patterns could be discerned. Consistent involvement was seen only in inguinal nodes of WTD and bronchial, inguinal, and mediastinal nodes of MD.

Lesions in the spleen involved activation of lymphoid germinal centers. The spleens of five of seven WTD exposed to worms were rated minor activity; two were rated as heavy activity. Of the three MD examined, splenic activity of two was rated moderate and one heavy. Three of four control

animals had inactive spleens; one animal had minor splenic activity.

Other organs

Additional gross lesions were seen in the gastro-intestinal tract and associated mesenteries. Areas of white fibrous material around an elongated (10X20 mm), raised, solid core were found on the serosal surface of the liver in one animal (MD 38) and abomasum of another (MD 39). Numerous round (10 mm diam.), hard, white nodules were seen throughout the mesentery of WTD 63. Cut surface of these nodules revealed an oedematous yellow granular center surrounded by a 2-3 mm thick homogeneous white wall. Identifiable structures were not found within these lesions.

An accumulation of soft, yellow material was noted around a degenerate worm in the omentum adjacent to the greater curvature of the abomasum and in the mesentery associated with the duodenum in WTD 51. Histologically, degenerate worms in the mesentery were surrounded by numerous eosinophils, plasma cells, and fibroblasts. A few giant cells adjacent to the cuticle and a diffuse infiltration of plasma cells and eosinophils in surrounding tissue were noted.

In WTD 41, frank haemorrhages were noted in mesenteries and perirenal fat, the intestine was oedematous and haemorrhagic, and approximately 10 cc of thin yellow fluid were found in the pericardial sac. A large 30X70 mm

haemorrhage involved all layers of the lesser curvature of the abomasum. No worms or evidence of worm movement were apparent in this haemorrhage; however, 47 adult worms were recovered from connective tissue of the adjacent fat. A few free erythrocytes were present in the fat.

The major histopathologic lesion noted in the duodenum was sloughing of surface epithelium from villi. Capillaries were congested and dilated and the lamina propria contained many plasma cells and a few lymphocytes and macrophages. Other lesions noted were increased activity in goblet cells, haemorrhage in the mucosa, increased connective tissue in the submucosa, and dilated central lacteals. All lesions were minor and indicative of a mild enteritis. No lesions were noted in duodenal sections of control fawns.

Mild inflammation and hyperplasia of lymphoid tissue were noted in abomasal mucosa of WTD and MD exposed to *P. andersoni*. Lesions were particularly common in the lesser curvature of the pyloric region. Mild oedema and congestion were observed in the submucosa, muscularis, and serosa of some deer. No lesions were present in control animals.

Focal hepatitis was seen in three WTD exposed to *P. andersoni*. Degenerate hepatocytes were noted around central veins in WTD 41.

Minor haemorrhage was the only gross lesion associated with eggs and adult worms recovered from the epidural space of the vertebral canal. No response was noted around worms in connective tissue of fat within the pelvic girdle.

It should be noted that most fawns examined in this study harbored low-level infections of trichostrongylid nematodes in the gastro-intestinal tract. Mean intensities were extremely low (<50 worms). Two WTD also harbored a few (<5) *Dictyocaulus viviparus* in the bronchi. No pathologic effects were apparent in any of these infections.

DISCUSSION

Lesions associated with *Parelaphostrongylus andersoni* infections involved localized insult to muscles, lungs and lymph nodes, and a generalized lymphoid hyperplasia. A mild enteritis and some loss of overall condition were also noted. In most animals, insult was considered minor in all areas except the lungs.

Damage in the skeletal muscles can be directly related to the activities of adult worms and the presence of eggs and larvae. Movement of adult worms through muscle tissue and penetration of veins and venules by female worms (Chapter III) damage intramuscular blood vessels and result in haemorrhage and subsequent necrosis. Furthermore, tissue damage was progressive throughout the infection. Some damage may also be associated with feeding by adult worms. Although red blood cells were commonly found in the gut of worms, few worms were found completely or with the anterior end directly within vessels (Chapter III). Thus, it is unlikely that worms feed directly from vessels. The intramuscular haemorrhages may serve as feeding sites. Additional damage

was associated with eggs and larvae. They appeared to provide a persistent insult to skeletal muscles and were always associated with extensive damage due to the inflammatory response.

Lesions in the lungs of infected animals are the result of filtration of eggs and larvae in the capillaries and are similar to those reported in infections of other species of *Parelaphostrongylus* (Hobmaier 1937, Anderson 1963). However, only the pathogenesis of *P. odocoilei* in the lungs of black-tailed deer (*O. h. columbianus*) has been described (Hobmaier 1937). Degenerative changes in *P. andersoni* infections appear to follow the same pattern. Eggs apparently lodge in capillaries within the alveolar walls and disrupt blood flow through the vessel. Collateral blood vessels attempt to restore blood flow through the area. Continued development of eggs within the capillary is associated with increased size of the egg and distortion of the capillary wall. In conjunction with increased blood pressure, the distortion may lead to rupture and haemorrhage into the adjacent parenchyma. In addition, a damaged capillary is more likely to receive further embolization due to its increased size and altered blood flow patterns. Thus, eggs may accumulate within such areas and repair is hampered by constant insult.

Tissue damage promotes activation of intrapulmonary lymphoid tissue, a localized accumulation of inflammatory cells, and subsequent granuloma formation. Tissue adjacent

to the granuloma becomes consolidated. Inflammation of airways and blood vessels disrupts normal flow patterns and results in emphysema and further haemorrhage.

Egg emboli carried to the lungs are filtered out in a generalized pattern throughout all lobes. Similar distribution of lesions is reported in *Skrjabingylus* infection in mustelids (Lankester and Anderson 1971). These infections lack the discrete localization seen in lesions caused by other lungworms such as *Protostrongylus* spp. of bighorn sheep (*Ovis canadensis*) (Pillmore 1961) and Dall sheep (*O. dalli*) (Neiland 1972), *Muellerius*, *Cystocaulus*, and *Protostrongylus* of domestic sheep (Beresford-Jones 1971), *Filaroides* of dogs (Hirth and Hottendorf 1973), and *Angiostrongylus* of shrews (Ogren 1954). The difference in pattern may relate to the site of the adult worms; i.e., extra vs. intrapulmonary. Eggs from parasites in extrapulmonary sites reach the lungs via the blood stream and are thus filtered in a generalized pattern throughout the lung. Eggs from parasites within the lungs are localized at the site of adult worms. The location of discrete lesions has been used to characterize the severity of *Protostrongylus* spp. infections (Pillmore 1961); however this is not possible for those species resulting in a diffuse distribution of lesions.

Specific insult to lymph nodes also involves filtration of eggs and larvae. Some eggs released directly into muscle tissue apparently enter lymphatics and are carried to the

regional lymph nodes. It is unknown how or where entry into lymphatics occurs. Lesions in a variety of nodes suggests it may occur anytime during the movement of eggs and larvae to the lungs. Consistent findings in inguinal and bronchial nodes indicate the majority are filtered out in the muscles of the hind quarters and in the lungs.

In a previous report (Nettles and Prestwood 1976), the primary lesions in two WTD exposed to 1000 larvae of *P. andersoni* were eosinophilic myositis associated with focal necrosis and mineralization. Gross lung lesions were not apparent and granulomas were seen only in one of the two deer. Lesions in deer exposed to moderate numbers of worms (200-356 larvae) were considered mild and not significant (Nettles and Prestwood 1976). Lesions noted in the current study were more severe at both dosage levels. The reasons for the differences are unknown. As shown in other host-parasite relationships, genetic differences between the source larvae (Basch 1975, Belosovic and Dick 1980) or between host populations (Wakelin 1978) may be responsible for different levels of pathogenicity. In addition, results may reflect differences in methodology between the two studies.

Host response to infection with *P. andersoni* is characterized by granulomatous inflammation around eggs and larvae. Granuloma formation is an attempt to sequester antigens of low diffusability and localize them at one site. In current infections, cellular makeup and progression of

developing granulomas are not typical of foreign body granuloma reactions but are consistent with formations of immunologic origin (see Lichtenberg 1962).

In general, the response of deer to infection with *P. andersoni* was moderate, however, there were three exceptions:

WTD 41 appeared to have been particularly susceptible to infection and failed to mount an effective immune response to the worms. It was the only WTD fawn to exhibit overt clinical signs and notably severe gross lesions. Worm establishment was abnormally high for *P. andersoni* infection in WTD (Chapter III) but, as results from this study indicate, cellular response was minimal. Relative to other WTD fawns, large numbers of first-stage larvae were observed in lymphatics. The lack of eosinophils in peripheral blood samples from WTD 41 may suggest an incomplete immune system in this individual.

In contrast, WTD 38 and 63 may have been particularly resistant to infection. Gross lesions were reduced and cellular response to all parasite stages was extensive. This reaction is indicative of a strong immunological response.

Further evidence suggesting involvement of an immunologic role in *P. andersoni* infections has been reported (Nettles and Prestwood 1976, Prestwood and Nettles 1977). These authors compared fawns exposed to single versus repeated doses and concluded that the latter method evoked an active immune response capable of limiting the infection.

In current data, response to *P. andersoni* infection in the two "resistant" deer also appeared successful in limiting the infection. Recovery of adult worms was low and eosinophilic myositis was associated with degenerate worm stages. Similar lesions are described in four WTD exposed to 1000 or 5000 larvae of *P. andersoni* (Nettles and Prestwood 1976). These authors conclude the response is dose dependent. The data from the present study suggest that host response may also be related to variation in host ability to respond to the insult.

The relationship between *P. andersoni* and the definitive host appeared to vary at the level of host individual and, thus, may be determined by the host rather than the parasite. Implicit in such a relationship is the possibility of overwhelming an individual incapable of suppressing the infection. Thus, *P. andersoni* could act as a factor in affecting selective host survival.

Four factors involved in determining pathogenicity of metastrongyles in the definitive host have been suggested [Anderson 1971, p.82]. Only two of these factors, number and position of worms, and suitability of the host-parasite relationship are applicable to data in this study. The extent of damage in infected fawns increased with increasing number of worms recovered and therefore supports the first suggestion. Many of the lesions noted in the current study appear to be common to most lungworm infections (see Stockdale 1976) and immunologic response similar to that

suggested herein has been described for *Dictyocaulus* infections in cattle (Jarrett et al. 1957), *Protostrongylus* in bighorn sheep (Hudson 1970, Spraker 1979), and *Muellerius* in domestic sheep (Beresford-Jones 1967). In addition, strength and effectiveness of response may vary with immunocompetence of individual hosts (Spraker 1979). Thus, the second factor, suitability of the relationship, may relate directly to ability of the host to mount an effective immune response.

Lesions present in lungs of all infected animals were severe enough to result in loss of functional lung tissue. It is unknown how much the respiratory capacity of infected animals was altered but the type and extent of lesions strongly implied interference with gaseous exchange throughout much of the lung. Extrapolating from 2-dimensional measurement of pulmonary lesions, consolidation of lung tissue averaged 17-47% in infected WTD and MD. The volume of lung impaired by response to *Protostrongylus* spp. infections averaged 8.3% and 7.3% of each set of lungs of male and female dall sheep, respectively (Neiland 1972). However, as indicated previously, pulmonary lesions in *Protostrongylus* infections are localized within the lungs. In an animal which often relies on speed and endurance to outrun its predators, the type and potential extent of lung damage seen in *P. andersoni* infections is considered sufficient to affect the outcome of the predator-prey relationship.

Parelaphostrongylus andersoni infections appeared to cause slightly more damage in MD than WTD. Percentage of initial dose recovered was higher in MD than WTD (Chapter III). Although gross muscle lesions were similar in both hosts, most other aspects of the host-parasite relationship evaluated in the current study were more severe in all MD and appeared to relate to a difference in immunologic response. In MD, there was an active immune reaction but it was not effective in destroying the parasites. The result was excessive damage to tissues with little effect upon the number of worms present. WTD appeared to be more efficient at limiting the infection as the response was moderated and damage, thus, reduced. The parasite may present a potential problem in MD populations but this potential is greatly reduced in WTD populations.

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LITERATURE CITED

Anderson, R.C. 1963. The incidence, development, and experimental transmission of *Pneumostrongylus tenuis* Dougherty (Metastrongyloidea: Protostrongylidae) of the meninges of the white-tailed deer (*Odocoileus virginianus borealis*) in Ontario. Can. J. Zool. 41:775-792

-----, R.C. 1971. Lungworms. *In*: Parasitic diseases of wild mammals, Davis and Anderson (eds.), Iowa State Univ. Press, Ames. pp. 81-126.

Anderson, R.C. and U.R. Strelive. 1967. The penetration of *Pneumostrongylus tenuis* into the tissues of white-tailed deer. Can. J. Zool. 45:285-289.

Basch, P.F. 1975. An interpretation of snail-trematode infection rates: specificity based on concordance of compatible phenotypes. Intern. J. Parasitol. 5:449-452

Belosovic, M. and T.A. Dick. 1980. *Trichinella spiralis*: comparison with an arctic isolate. Exper. Parasitol. 49:266-276

Beresford-Jones, W.P. 1967. Observations on *Muellerius capillaris* (Muller, 1889) Cameron, 1927. Res. Vet. Sci. 8:272-279

-----, 1971. Pathology and parasitology of sheep lungworm infections in lungs and mediastinal lymph nodes. *In*:Pathology of Parasitic Diseases. Proc. 4th I. Internat. Conf. Wld. Assoc. for the Adv. of Vet. Parasitol., Gaafar (ed.), Purdue Univ. Studies, Lafayette. pp. 287-294.

Cottier, H., J. Turk, and L. Sobin. 1972. A proposal for a standardized system of reporting human lymph node morphology in relation to immunological function. Bull. Wld. Hlth. Org. 47:375-408

Hirth,R.S., and G.H. Hottendorf. 1973. Lesions produced by a new lungworm in beagle dogs. Vet. Pathol. 10:385-407

Hobmiaer, M. 1937. Studies on the pathology of *Elaphostrongylus odocoilei* in *Odocoileus columbianus columbianus*. Raboty Gel'mint. pp 235-240

Hudson, R.J. 1970. Immunology of lungworm (*Protostrongylus*) infections of the Rocky Mountain bighorn sheep. Ph.D. Thesis, Univ. British Columbia, Vancouver, B.C. 170 pp.

Jarrett, W.F., W.I. McIntyre, G.M. Urquhart. 1957. The pathology of experimental bovine parasitic bronchitis. J. Pathol. Bact. 73:183-193

Lankester, M.W. 1977. Neurologic disease in moose caused by *Elaphostrongylus cervi* Cameron 1931 from caribou. Proc. N. Am. Moose Conf. and Workshop 13:177-190

Lankester, M.W. and R.C. Anderson. 1971. The route of migration and pathogenesis of *Skrjabingylus* spp. (Nematoda: Metastrongyloidea) in mustelids. Can. J. Zool. 49:1283-1293

Lichtenberg, F.v. 1962. Host response to eggs of *S. mansoni*. I. Granuloma formation in the unsensitized laboratory mouse. Am. J. Pathol. 41:711-723

Lichtenberg, F.v. and S. Mekbel. 1962. Granuloma formation in the laboratory mouse. 1. Reaction to *Ascaris suis* eggs in the unsensitized adult and newborn. J. Infect. Dis. 110:246-252

Neiland, K.A. 1972. Sheep disease studies. Alaska Dept. Fish and Game, Juneau. Project Progress Rpt, Project W-17-3 and W-17-4

Nettles, V.F. and A.K. Prestwood. 1976. Experimental *Parelaphostrongylus andersoni* infections in white-tailed deer. Vet. Pathol. 13:381-393

Ogren, R.E. 1954. A lungworm, *Angiostrongylus blarini*, n. sp., from the short-tailed shrew, with observations on the histopathology and life cycle. J. Parasitol. 40:681-685

Pillmore. R.E. 1961. Investigations of diseases and parasites affecting game animals. 1. Comparative studies of infection intensities in wild and laboratory

populations. Fed. Aid Rpt. W-95-R-4. Colo. Div. Wildl. pp 83-97

Prestwood, A.K. 1972. *Parelaphostrongylus andersoni* sp. n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus virginianus*). J. Parasitol. 58:897-902

Prestwood, A.K. and V.F. Nettles. 1977.Repeated low-level infection of white-tailed deer with *Parelaphostrongylus andersoni*. J. Parasitol. 63:974-978

Prestwood, A.K., V.F. Nettles, and F.E. Kellogg. 1974. Distribution of muscleworm, *Parelaphostrongylus andersoni*, among white-tailed deer of the southeastern United States. J. Wildl. Dis. 10:404-409

Pursglove, S.R. 1977. Helminth parasites of white-tailed deer (*Odocoileus virginianus*) from New Jersey and Oklahoma. Proc. Helminthol. Soc. Wash. 44:107-108

Pybus, M.J. and W. M. Samuel. 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. 45:537-542

Spraker, T.R. 1979. The pathogenesis of pulmonary protostrongylosis in bighorn lambs. Ph.D. Thesis, Colo. State Univ., Fort Collins, Colo. 233 pp.

Stockdale, P.H. 1976. Pulmonary pathology associated with metastrongyloid infections. Br. Vet. J. 132:596-608

Stockle, A.W., G.L. Doster, and W.R. Davidson. 1978. Endogenous fat as an indicator of physical condition of southeastern white-tailed deer. Proc. Ann. Conf. S.E. Assoc. Fish and Game Agencies. 32:269-279

Wakelin, D. 1978. Genetic control of susceptibility and resisitance to parasitic infection. Adv. Parasitol. 16:219-308

V. Pathology of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in two cervid hosts.

ABSTRACT

Pathologic effects and host response were evaluated in seven white-tailed deer (*Odocoileus virginianus*) and six mule deer (*O. hemionus hemionus*) each exposed *per os* to 300 or 1000 third-stage larvae of *Parelaphostrongylus odocoilei*. Pathologic effects in mule deer consisted of haemorrhagic myositis throughout skeletal muscles, severe verminous pneumonia, and moderate lymphadenitis. The major host response was a granulomatous inflammation associated with nematode eggs and larvae. Granulomas obliterated the normal architecture of affected tissues. Pathologic effects and host response were minimal in white-tailed deer. *P. odocoilei* is considered a potential direct or indirect pathogen in mule deer but an insignificant parasite in white-tailed deer.

INTRODUCTION

Natural infections of *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier 1934) are reported only from black-tailed deer, *Odocoileus hemionus columbianus* and/or mule deer, *O. h. hemionus* (MD) in California (Hobmaier and Hobmaier 1934, Brunetti 1969) and Alberta (Platt and Samuel 1978). Although specific identification of *P. odocoilei* is

possible only on the basis of morphology of adult worms, a high prevalence of eggs and larvae indistinguishable from those of *P. odocoilei* (Brunetti 1969, Samuel unpub.) suggests that the worm may be a common parasite of *O. hemionus*.

Within the definitive host, adult worms apparently move through connective tissue in a variety of skeletal muscles and associated fat deposits (Chapter III). Although some eggs and larvae are found in host lymphatics (Hobmaier and Hobmaier 1934, Brunetti 1969, Chapter III), most are released directly into the circulatory system and filtered out in capillary beds in the lungs (Hobmaier 1937, Chapter III). Lesions in naturally-infected deer (Hobmaier and Hobmaier 1934, Hobmaier 1937, Brunetti 1969) suggest that the potential exists for the worm to cause damage to vital tissues of the host. Indeed, *Parelaphostrongylus odocoilei* was considered the primary cause of death in a yearling black-tailed deer (Brunetti 1969) and could be an important direct or indirect mortality factor in host populations.

Odocoileus hemionus appears to be the primary host species for this worm. However, in many parts of its range, *O. hemionus* is sympatric with other potential cervid hosts, such as, white-tailed deer (*O. virginianus*)(WTD), wapiti (*Cervus elaphus*), and moose (*Alces alces*). The pathologic effects and host response in these species have been examined only in moose. Extensive damage in the lungs and skeletal muscles of experimentally-infected calves is

reported (Pybus and Samuel 1980). This paper reports the pathologic effects and host response to *P. odocoilei* in experimentally-infected captive MD and WTD fawns.

MATERIALS AND METHODS

In 1978-1981, neonatal MD and WTD fawns were collected from various regions of Alberta. Animals were maintained at the University of Alberta Biomedical Animal Centre following procedures as outlined in Chapter II. After weaning, six MD and seven WTD approximately 12 weeks old were exposed *per os* to 300 or 1000 third-stage larvae of *P. odocoilei* (Table I). Larvae were derived from natural infections in MD in Jasper National Park, Alberta. Two MD and four WTD were maintained as control animals.

Further methods were identical to those detailed in Chapter IV. Briefly, animals were weighed and bled weekly starting 2-3 weeks prior to exposure. Total RBC, total WBC, hematocrit, and haemoglobin values were determined. Two-way analysis of variance of hematologic data grouped by host (MD or WTD) and treatment (infected or control) in each of three time periods (pre-exposure, pre-patent, post-patent) was followed by pairwise comparison of group means. Time periods in control animals were derived from mean values in fawns exposed to worms. Regression slopes of linear increase in weights were compared.

Animals were killed by intravenous injection of sodium pentabarbitol at different times after exposure to worms

Table I. Experimental design to investigate the pathology of *Parelaphostrongylus odocoilei* in fawns.

	Mule deer				White-tailed deer				Control
	Deer number	Dose	dPE*	Worm recovery**	Deer number	Dose	dPE	Worm recovery	Deer number
1978	MD 14	300	104	51	WTD 28	300	580	0	WTD 26
	MD 20	300	138	38	WTD 29	300	124	0	WTD 30
	MD 21	300	99	38					
1979	MD 26	300	121	53	WTD 37	300	83	0	WTD 40
					WTD 44	1000	69	0	WTD 43
					WTD 45	300	107	0	MD 30
					WTD 46	1000	75	0	
					WTD 50	300	93	0	
1981	MD 41	300	126	38					MD 36
	MD 55	300	54	54					

* Days post exposure when animal killed.
** Expressed as percentage of dose.

(Table I). Assessment of body condition was based on the amount and extent of subcutaneous and visceral fat using five categories ranging from poor to excellent. At necropsy, particular attention was given to the skeletal muscles, lungs, central nervous system, and vertebral canal. The color, texture and size of the lungs was assessed and ranked on a scale of 1 to 5 (normal to severe). Percentage of consolidated lung parenchyma as well as the size and density of granulomas within histologic sections of the lungs were determined. The size and number of germinal centers, cellularity of medullary sinuses, and size of medullary cords in the lymph nodes and spleen were assessed and grouped into four categories (inactive to heavy activity). Individual nodes were ranked by the activity of the node and the number of deer in which a node was affected. Quantitative data were analyzed by ANOVA. A significance level of $p<0.05$ was accepted for all analyses.

RESULTS

Clinical signs and hematology

No animals exhibited clinical signs prior to 75 days post-exposure (dPE). At 75 dPE, all infected MD became generally inactive and dyspnoea was noted after minimal exercise. One of these fawns developed continual laboured breathing 92 dPE and minor exercise resulted in prolonged gasping. This animal died in respiratory distress 104 dPE (7 wks post patency). Much haemorrhagic foam was discharged

from the mouth and nares at death. Control animals and WTD exposed to *P. odocoilei* remained clinically normal throughout the observation period.

MD and WTD exposed to *P. odocoilei* exhibited a significant increase in the percentage of eosinophils in differential WBC counts after exposure to larvae ($F(5,132)=12.14$, $F(5,168)=12.04$, respectively, $p<0.001$) (Table II). Absolute eosinophil values also increased after fawns were exposed ($F(5,126)=8.61$, $F(5,157)=5.91$, $p<0.0001$, MD and WTD, respectively). After patency, levels were maintained in MD but returned to control levels in WTD. No differences were noted between either MD or WTD control values in different time periods. All other hematologic values were similar in infected and control animals (Table II).

General condition

Body condition of control animals was excellent in three WTD (WTD 26,40,43) and very good in one MD (MD 30) and one WTD (WTD 30). Of six MD exposed to *P. odocoilei*, one was rated in very good condition (MD 41), three were good (MD 20,26,55), one was fair (MD 21), and one was poor (MD 14). Of six WTD exposed to *P. odocoilei*, condition was excellent in three animals (WTD 29,37,44), very good in one (WTD 45), and good in two (WTD 46,50). Note, of the two deer evaluated as good, one had a congenital locomotory problem and the other did not adapt well to captivity and appeared stressed

Table II. Haematologic values of fawns exposed to *Parelaphostrongylus odocoilei* and of control animals.

	PCV (%)	Hgb (g/100ml)	RBC (10^6/mm^2)	WBC (/mm^2)	Differential WBC (%)				
					Neut	Bas	Eos	Lymph	Mono
Mule deer exposed to *P. odocoilei* (n=4)									
Pre-exposure	49±4*	18.0±1.6	11.6±1.2	4869±13	48±10	1±1	2±2	44±9	4±3
Pre-patent	47±6	17.2±2.5	11.8±2.1	6382±2033	47±11	2±2	6±4	42±10	3±2
Post-patent	38±3	14.8±1.3	10.4±1.3	7766±3421	40±11	6±4	8±5	43±13	3±3
White-tailed deer exposed to *P. odocoilei* (n=7)									
Pre-exposure	50±5	19.0±1.8	17.3±1.9	4154±2356	33±8	1±1	4±4	55±8	7±3
Pre-patent	47±6	17.8±2.3	17.7±2.2	5148±2256	35±11	2±2	9±9	49±11	5±3
Post-patent	42±3	15.7±1.2	16.1±1.6	4659±1485	39±13	1±1	3±3	54±12	3±3
Mule deer controls** (n=5)									
Pre-exposure	48±4	18.0±1.7	12.8±1.4	4718±1327	45±14	1±1	2±2	46±14	5±2
Pre-patent	46±4	17.4±1.7	12.5±1.3	5148±1158	45±13	0±1	3±3	48±11	4±2
Post-patent	44±5	16.1±1.9	11.6±1.3	5173±942	50±14	1±1	3±2	44±14	3±2
White-tailed deer controls** (n=4)									
Pre-exposure	50±5	19.0±1.7	16.6±3.3	4917±2369	43±15	1±1	2±2	49±17	6±4
Pre-patent	47±5	18.0±1.7	17.7±1.7	4169±1318	38±19	1±1	1±1	55±19	5±3
Post-patent	43±4	16.2±1.8	16.2±2.3	4478±621	37±20	1±1	3±4	57±20	3±3

* Mean ± S.D

** Time periods determined using mean values of infected deer.

throughout the experiment.

Weight gains throughout the experimental period did not differ between years nor between infected and control animals.

Muscle pathology

All worms recovered from MD were associated with gross tissue damage; however, the size and extent of damage varied with length of infection. At 54 dPE, the characteristic lesions were focal necrosis and numerous small areas (1-2 cm^2) of thick black blood pooled between muscle fibres. Focal accumulations of thick yellow material were seen on nerves in the right semimembranosus, right psoas, and left longissimus dorsi.

At 99 and 104 dPE, haemorrhage varying from small localized areas (5-15 mm diam.) to extensive paintbrush lesions (30 mm diam. X 70 mm length) were noted. Worms were found partially or entirely within the intramuscular haemorrhages. Occasionally, long thin haemorrhages (10 mm diam. X 20-40 mm length) were found, usually with worms at one end of the haemorrhage. Muscle fibres were friable. Focal necrosis was prominent throughout muscles of the hind quarters.

At 121-138 dPE, extensive frank haemorrhage and focal necrosis was seen in skeletal muscles. Long, thin haemorrhages were common in muscles of the back and thighs.

Adult *P. odocoilei* were found only in areas of haemorrhage. A few degenerating worms ensheathed with thick yellow-white material were seen on the epimysium or deep within muscle bundles. Adjacent muscle tissue did not appear to be affected.

Histologic examination of infected MD revealed diffuse haemorrhage associated with minor cellular response throughout muscle sections (Fig. 1-4). Moderate numbers of eosinophils and a few small lymphocytes were seen in tissue directly adjacent to adult worms. Muscle fibres were pale and swollen with occasional calcified degenerate fibres.

Vasculitis was prominent in most muscle sections. Vessels (usually veins and venules) were seen in various stages of degeneration as vascular congestion, occlusion, collapse, distension, and occasional cuffing with lymphocytes were all noted. Female worms in veins often were at the core of a thrombus consisting of eosinophils, lymphocytes, and macrophages within a fibrin matrix.

In later infections, lesions were more extensive and associated with eosinophils and macrophages. Eggs and larvae in fat, connective tissue, and muscles adjacent to veins were associated with lymphocytes, macrophages, giant cells and necrosis (Fig. 5,6).

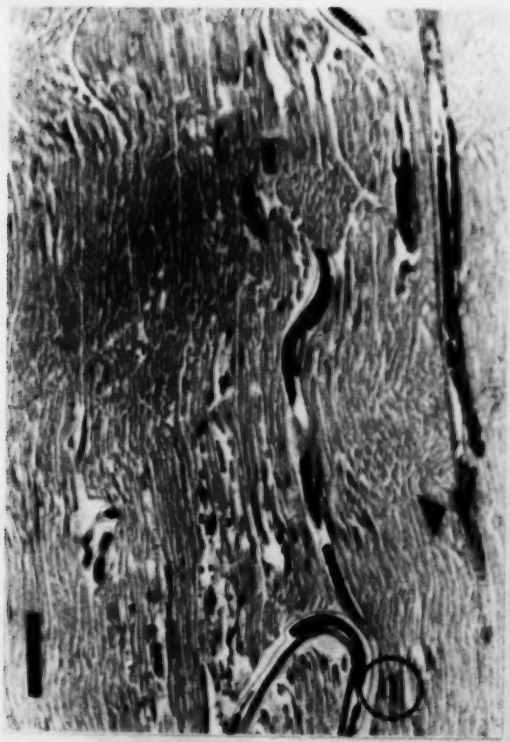
Lesions adjacent to peripheral nerves were associated with the presence of nematode eggs and first-stage larvae. Localized accumulations of neutrophils and lymphocytes were seen in the connective tissues and fat adjacent to nerves.

Figure 1. Minor haemorrhage (▲) and cellular response to adult Parelaphostrongylus odocoilei in the biceps femoris of a MD. Bar = 660 um

Figure 2. Vasculitis associated with a female Parelaphostrongylus odocoilei in a vein in the longissimus dorsi of a MD. Little or no response to the worm in the adjacent muscle tissue. Bar = 410 um

Figure 3. Cross-section of an adult Parelaphostrongylus odocoilei in the abdominal wall of a MD. A few inflammatory cells are directly adjacent to the worm. Bar = 57 um

Figure 4. Extensive haemorrhage associated with an adult Parelaphostrongylus odocoilei in the longissimus dorsi of a MD. Bar = 66 um



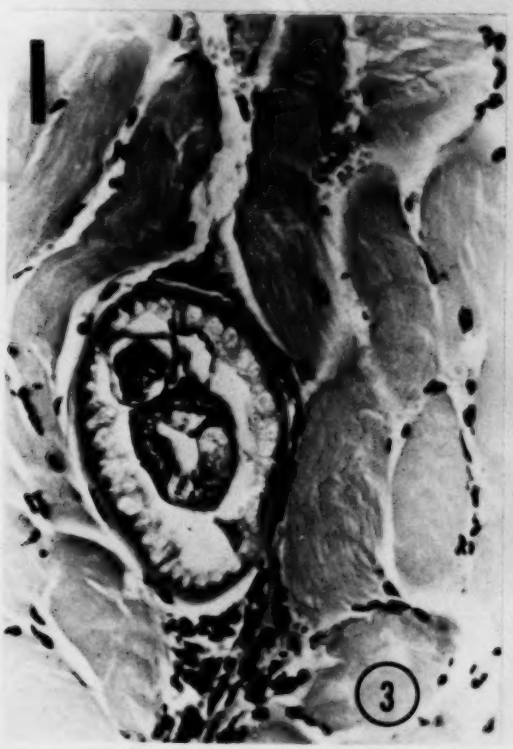


Figure 5. Granulomatous inflammation associated with eggs and larvae of Parelaphostrongylus odocoilei in the fat of a MD. Bar = 268 um

Figure 6. Granulomatous inflammation associated with eggs and larvae of Parelaphostrongylus odocoilei in the gluteus major of a MD. Bar = 268 um

Figure 7. Larvated eggs of Parelaphostrongylus odocoilei in the lung of a MD. Bar = 63 um

Figure 8. Granulomas associated with eggs and larvae of Parelaphostrongylus odocoilei in the lungs of a MD. Bar = 500 um

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Some cells were within the endoneurium directly adjacent to nerve fibres but the fibres appeared to be unaffected. First-stage larvae were associated with giant cells and minor swelling and vacuolation of some nerve fibres.

Lesions were not noted in the skeletal muscles of WTD exposed to *P. odocoilei* or of control animals.

Lung pathology

The lungs of both control MD and three of four control WTD were grossly normal. The remaining WTD had minor damage consisting of a few petechial haemorrhages in one diaphragmatic lobe. In MD exposed to *P. odocoilei*, the lungs of one animal were heavily damaged and those of five were severely damaged. Cut surface of the lungs of all infected MD revealed a firm parenchyma with extensive white fibrous tissue interspersed with focal haemorrhage. In WTD exposed to *P. odocoilei*, the lungs of four animals were normal and those of three had minor lesions.

Mean total lung weights were significantly higher in the six infected MD (1085$\pm$141g) than in control MD (280, 360g) ($F(1,5)=43.60$, $p<0.002$) and five infected WTD (297$\pm$109g) ($F(1,8)=87.08$, $p<0.0001$). No other significant differences were noted. The lungs of the two remaining WTD were not weighed.

The major histologic lesion in the lungs of MD was a granulomatous inflammation around eggs and larvae within the alveolar walls (Fig. 7,8). Granulomas were distributed

throughout all lobes of the lung and additional lesions were related to the size and number of granulomas present (Fig. 9-11).

In early infections, a few large mononuclear cells were present within the alveolar walls containing an egg or larva. These small, discrete granulomas were most common in tissue adjacent to respiratory or terminal bronchioles in peripheral areas of the lobules. Caseous necrosis was noted in the central areas of a few granulomas.

Generalized congestion and thickening of alveolar walls was apparent throughout lobules containing eggs and/or larvae. A mild bronchitis and bronchiolitis was associated with larvae in the lumen or mucosa of small airways. Neutrophils and a few eosinophils were present in bronchial lumina. Occasionally, thrombi of eggs, neutrophils, and macrophages were present. Lobules without parasites were mildly emphysematous.

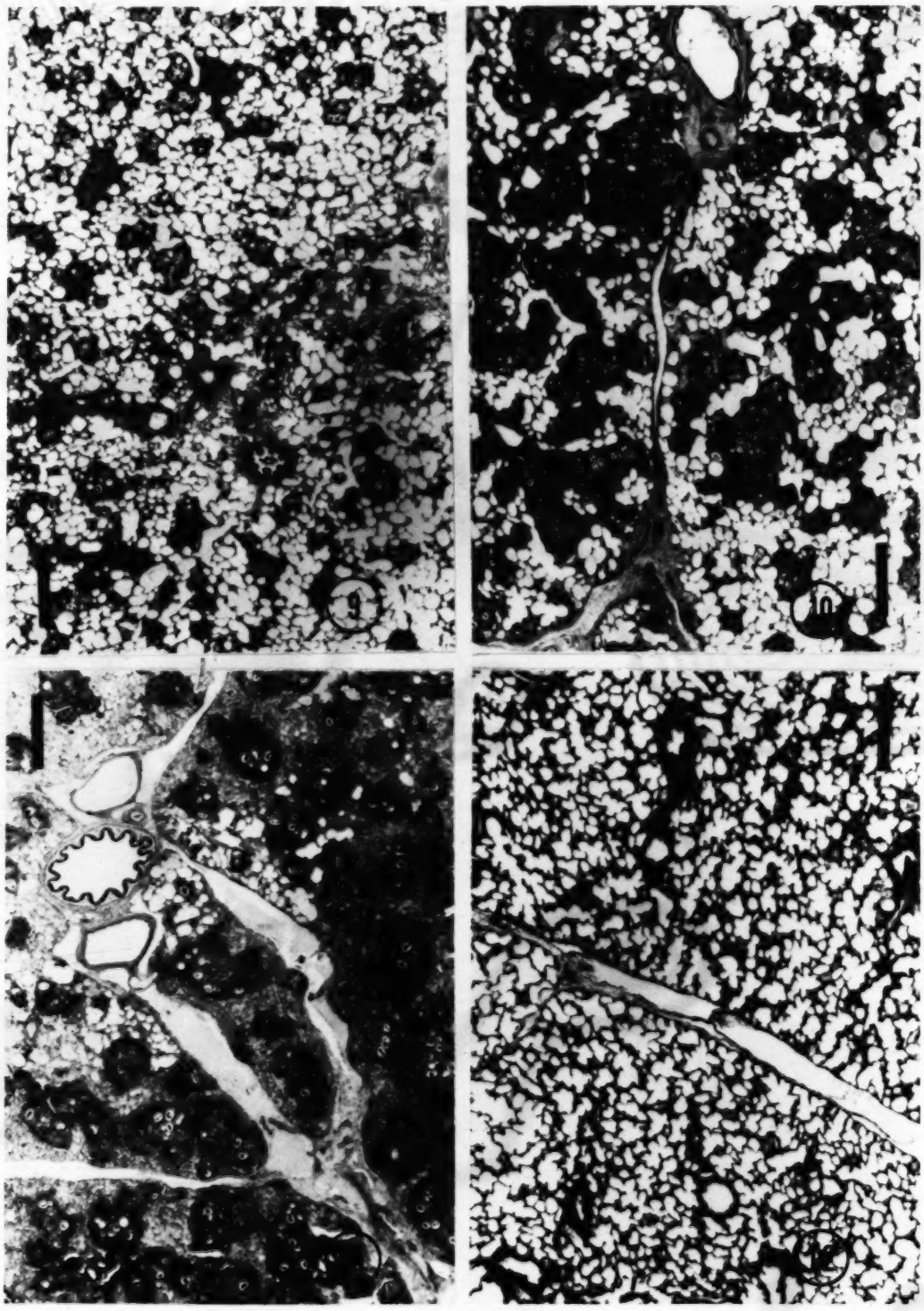
All lesions present in the early stages increased in severity throughout the infection. Granulomas, consisting of large mononuclear cells and a few neutrophils and eosinophils became large and confluent, completely destroying the normal architecture of the parenchyma. Fibroblasts were noted on the periphery of granulomas. Most respiratory bronchioles were obliterated by granulomas but other granulomas were widely distributed throughout all lobes.

Figure 9. Small discrete granulomas distributed throughout the lung of a MD exposed to Parelaphostrongylus odocoilei. Bar = 575 um

Figure 10. Moderate-sized granulomas associated with atelectasis and emphysema in a MD exposed to Parelaphostrongylus odocoilei. Bar = 575 um

Figure 11. Large, confluent granulomas associated with extensive atelectasis, haemorrhage, and oedema in a MD exposed to Parelaphostrongylus odocoilei. Bar = 575 um

Figure 12. Lungs of a control animal. Bar = 575 um



Focal necrosis and calcification were seen commonly in granulomas containing hatched eggs and/or first-stage larvae. Necrotic areas were surrounded by extensive localized haemorrhage and pink proteinaceous fluid free within airways and alveolar spaces. Bronchi and bronchioles were dilated in all sections and many airways contained larvae, lymphocytes, neutrophils, mucous and cellular debris. Alveolar spaces not directly involved in the granulomatous response were distended and emphysematous. Inflammation of interstitial tissues was also present.

Extensive vasculitis, particularly arteritis, was noted in all lung sections. Lesions consisted of perivascular cuffing with small and large lymphocytes, vascular congestion and occlusion with larvae and cellular debris, and degeneration of arterial walls.

Histologic lesions in controls and WTD exposed to *P. odocoilei* did not differ and were indicative of a mild inflammatory response to persistent irritation, possibly, dust (Fig. 12).

Consolidation was present in the lungs of all animals examined. Quantitatively, percent consolidation was significantly greater in MD exposed to *P. odocoilei* than in control animals ($F(2,36)=71.38$, $p<0.0001$) and WTD exposed to worms ($F(2,40)=27.57$, $p<0.0001$). Values were also higher in WTD exposed to worms than in controls ($F(2,28)=11.45$, $p<0.002$). The mean size of pulmonary granulomas (seen only in MD exposed to worms) differed significantly among fawns

($F(5,1178)=197.86$, $p<0.0001$) but the density of granulomas was similar ($F(5,18)=1.35$, $p>0.05$) (Table III).

Lymph node and spleen pathology

Generalized lymphadenosis was present in all MD exposed to *P. odocoilei*. Haemorrhage and inflammation were consistently seen only in deep inguinal nodes.

Gross lesions were not seen in the lymph nodes of control animals or of WTD exposed to *P. odocoilei*.

Histopathologic evaluation of the lymph nodes of four MD exposed to *P. odocoilei* revealed granulomas in the afferent lymphatics, subcapsular spaces, and distal cortex of a variety of nodes in all deer (Table IV). Granulomas consisted of a core of eggs and larvae surrounded by giant cells, macrophages and pink amorphous material. In two deer, eosinophils were also common in granulomas. Fibroblasts and loose connective tissue were noted on the periphery of the granulomas. Granulomatous inflammation appeared to be more common in later infections.

Arteritis, congestion, haemorrhage, and siderocytes were common in the medullary sinuses of affected nodes. Germinal centers were hyperplastic in all nodes containing parasites. Minor insults of consolidation and oedema within the medullary sinuses were also noted in various nodes.

Lesions in the lymph nodes of control animals and of WTD exposed to *P. odocoilei* were mild congestion and accumulation of haemosiderin in mesenteric and ruminal lymph

Table III. Quantitative histopathology of lungs of white-tailed deer (WTD) and mule deer (MD) fawns exposed to *Parelaphostrongylus odocoilei* and of control fawns.

	Percent consolidation	Mean granuloma size (um)	Granuloma density (No./mm^2)
MD 14	70±12*	371±37	2.1±0.6
MD 20	84±2	318±60	3.1±0.8
MD 21	48±20	190±41	3.3±2.0
MD 26	31±8	260±30	2.5±0.3
MD 41	27±10	176±14	4.1±1.3
MD 55	28±21	121±5	3.5±1.6
WTD 29	12±2	-	0
WTD 37	16±7	-	0
WTD 44	10±2	-	0
WTD 46	19±12	-	0
WTD 26**	2±2	-	0
WTD 30**	6±3	-	0
WTD 40**	15±10	-	0

* Mean for four lobes ± S.D.
** Control fawn.

Table IV. Histopathology of lymph nodes and spleen of control animals and of fawns exposed to *Parelaphostrongylus odocoilei*.

	Activity level			Number of nodes containing granulomas		
	Control	Mule Deer	White-tailed deer	Control	Mule deer	White-tailed deer
N	4	4	6	4	4	6
Axillary	inactive**	moderate	moderate	0	1	0
Bronchial	-	moderate	heavy	-	2	0
Inguinal	minor	heavy	moderate	0	2	0
Mediastinal	-	heavy	-	-	1	-
Mesenteric	moderate	moderate	variable	0	0	0
Ruminal	heavy	moderate	heavy	0	0	0
OVERALL	inactive	moderate	moderate-heavy	-	-	-
Spleen	inactive	moderate	moderate	-	-	-

* White-tailed deer only.
** Modal values. See text for details.

nodes. No granulomas, eggs, or larvae were seen.

The modal value of activity levels in lymph nodes and spleen was consistently higher in animals exposed to worms than in control animals (Table IV). Axillary, bronchial, and inguinal lymph nodes were most heavily affected.

Other organs

Verminous hepatitis was noted in one MD exposed to *P. odocoilei*. Grossly, a large (40X10X10 mm) firm, white area was present on the ventral edge of the left lobe of the liver. Histologically, numerous discrete round (mean size=532±255 um) granulomas, similar in make-up to those seen in the lymph nodes, were found in portal regions within the lesion. Eosinophils were common in these areas. Granulomas near the serosa appeared larger and contained more larvae than those in deeper tissue. The liver parenchyma was completely destroyed throughout the lesion while hepatocytes not directly involved in granulomas were large and vacuolated. Minor focal inflammation was present in some portal areas throughout the remainder of the liver. Additional hepatic lesions consisted of minor focal hepatitis in three MD and one WTD exposed to *P. odocoilei*.

A mild to moderate enteritis was present throughout the gastro-intestinal tract of all deer exposed to worms. Lesions were more extensive in MD. No lesions were seen in gastro-intestinal tissues of control animals.

Miscellaneous infections with helminths other than *P. odocoilei* (usually gastro-intestinal trichostrongyles) were present in many fawns. Intensities were low (<50 worms) and no pathologic effects associated with these infections could be determined. Two MD harboured low-level infections of *Taenia krabbei* in skeletal muscles. Response to these infections was minimal and restricted to the immediate vicinity of the cyst. Three MD and one WTD were infected with low numbers of *Dictyocaulus viviparus* in the bronchioles. Infections were not patent and damage to the lungs was minimal.

DISCUSSION

Host response to infection with *P. odocoilei* depended on the stage of development of the parasite. Cellular response to adult worms in connective tissue of skeletal muscles was minimal. In contrast, eggs and larvae evoked a granulomatous inflammation in all tissues where they were found. Components of the response indicated activation of the host immune system similar to the situation observed in *P.* andersoni infections (see Chapter IV). Different responses to different development stages have been described in other nematode infections and may relate to antigenic makeup (Schacher and Sahyoun 1967), metabolic activity (Love and Ogilvie 1975), or sequential development of an immune response (Rogers et al. 1975).

Pathologic insult was associated with all stages of *Parelaphostrongylus odocoilei* infections. Haemorrhage and necrosis were common throughout skeletal muscles and appeared to relate directly to the activity of adult worms. Large numbers of worms recovered from a variety of muscles in MD (Chapter IV) could cause widespread impairment of muscles in infected individuals. The absence of clinical anomalies in the gait and stance of infected animals may be associated with decreased activity of captive fawns; however, the muscle lesions observed are considered severe enough to interfere with movement in free-ranging deer.

Tissue disruption and subsequent necrosis and calcification were associated with all eggs and larvae seen in tissue sections. The granulomatous inflammation could lead to blockage of normal circulatory patterns in the lungs (blood and air flow) and lymphatics (lymph flow) and, thus, impair the functioning of these vital tissues. The respiratory distress noted in our captive, experimental animals would be compounded in free ranging animals with greater oxygen demands. The latter individuals could easily be compromised in their ability to respond to stresses and predators encountered in daily activities.

The actual effects of insults in the lymphatic system on the well-being of the host are unknown. Regional lymph nodes are important components of the immunologic response and provide a primary defence against soluble and particulate antigens. Lymph received from afferent

lymphatics is filtered as it drains through the cortex and medulla. Any disruption of this flow would impede the function of the node and, thus, compromise the immunologic competence of an animal.

The granulomatous response to eggs and larvae in lymph nodes could easily disrupt lymph circulation in MD infected with *P. odocoilei*. Although the lymph system does not appear to be a major route for eggs and larvae to reach the lungs (Chapter III), granulomas were a frequent insult in lymph nodes of infected MD and the number of granulomas appeared to increase in later infections. Thus, the possibility of damage to the lymphatic system, appears high.

Parelaphostrongylus odocoilei is a potentially serious pathogen in MD populations. Tissue damage was severe and the extent of insult increased throughout infection while evidence of repair was minimal. All infected MD exhibited a loss of overall condition during the infection and one fawn died as a direct result of respiratory difficulties. In addition, experimental infection with *P. odocoilei* has been maintained in MD for at least six years [Samuel, unpub. data]. Thus, damage to an infected deer could accumulate for long periods.

Information concerning natural infection levels is scanty and the dosage levels used in the current study are arbitrary. However, distinct trends in overall response are apparent. The data suggest that a few individuals in a MD population could be directly overwhelmed by infection with

P. odocoilei. In contrast, the majority of animals appear able to withstand the direct effects of an infection. But, regardless of initial dose, they will suffer progressive damage to vital tissues. Such damage would be an additional stress on a free-ranging host.

Damage to pulmonary, lymphatic and muscular tissue appears insignificant in WTD exposed to *P. odocoilei*. Although patent infections were established in WTD (Chapter III), reduced pathology and failure to recover larvae and adult worms at necropsy suggests low establishment rates and rapid removal of worms from the host. It is unknown why infection with *P. odocoilei* is not maintained in WTD nor at what stage the infection is limited.

Variation in pathogenicity is apparent in different host species exposed to *P. odocoilei*. Although the location and type of response are similar in MD to those in black-tailed deer and moose (Hobmaier and Hobmaier 1934, Pybus and Samuel 1980, respectively), severity of infection differs. It is low in WTD, moderate in moose, and high in MD (the severity of infection in black-tailed deer is poorly understood but probably is low to moderate [Pybus, unpub. data]). Differences appear directly related to numbers of adult worms present in skeletal muscles.

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LITERATURE CITED

Brunetti, O.A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game 55:307-316

Hobmaier, A. and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n. sp., a new lungworm in black tail deer (*Odocoileus columbianus*). Description and life history. Proc. Soc. Exp. Biol. Med. 31:509-514

Hobmaier, M. 1937. Studies on the pathology of *Elaphostrongylus odocoilei* in *Odocoileus columbianus columbianus*. Raboty Gel'mint. :235-240

Love, R.J. and B.M. Ogilvie. 1975. *Nippostrongylus brasiliensis* in young rats: lymphocytes expel larval infections but not adult worms. Clin. Exp. Immunol. 21:155-162

Platt, T.R. and W.M. Samuel. 1978. A redescription and neotype designation for *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea). J. Parasitol. 64:226-232

Pybus, M.J. and W.M. Samuel. 1980. Pathology of the muscleworm, *Parelaphostrongylus odocoilei* (Nematoda:

Metastrongyloidea), in moose. Proc. N. Amer. Moose Conf. and Workshop 16:152-170

-----, 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. 45:537-542

Rogers, R. D.A. Denham, G.S. Nelson, F. Guy, and T. Ponnudurai. 1975. Studies with *Brugia pahangi* III: Histological changes in the affected lymph nodes of infected cats. Ann. Trop. Med. Parasitol. 69:77-84

Schacher, J.F. and P.F. Sahyoun. 1967. A chronological study of the histopathology of filarial disease in cats and dogs caused by *Brugia pahangi* (Buckley and Edeson, 1956). Trans. Roy. Soc. Trop. Med. Hyg. 61:234-243

VI. Discussion

In any successful host-parasite relationship, a host must provide the proper anatomical, physiological, and biochemical conditions to allow development of the parasite (Kennedy 1975). At the same time, the host may respond to the parasite and act to limit the infection (Dogiel 1964, Kennedy 1975). Thus, the characters used to evaluate a host-parasite relationship are reflections of a complex interwoven balance of factors which facilitate and those which inhibit growth and development of the parasite (Lewis 1953, Garber 1956). The interactions of these factors differ between hosts and no two host species have equal susceptibility or tolerance to the same parasite (Barbehenn 1969).

Odening (1976) lists nine criteria which can be used to evaluate the success of a host-parasite relationship. Seven of these are applicable to the type of data collected using the classical experimental approach: rate of development of the parasite, intensity of infection (=establishment), longevity of the parasite, reproductive capacity of the parasite, transmissibility of parasite reproductive products, intensity of the host defence, and ability of the host to withstand insult.

In the traditional view, success is determined using the basic premise that a well-adapted relationship includes

high establishment rates and reproductive output of the parasite, rapid development and migration within the host, and a minimum of damage to the host (see Wakelin 1976, Holmes in press.). This classical view of host-parasite relationships will be used to evaluate current information concerning *Parelaphostrongylus andersoni* and *P. odocoilei* in WTD and MD.

Any host-parasite relationship can be evaluated in the individual host or the host species. Variation in the response to infection in individual hosts has been noted in numerous parasitic infections and was seen in WTD exposed to *P. andersoni*. Such variation has been attributed to genetic variability, age, sex, nutritional status, and reproductive status of the host (see Wakelin 1978). Animals in the current study were similar in age, nutritional status, and were all handled in a similar manner. However, they were collected from widely separate regions within Alberta and could easily represent genetic variation between populations as well as between individuals. In spite of variation between individuals, distinct patterns of host-parasite relationships could be determined for each host species.

A summary of the data from the experimental infections of *Parelaphostrongylus andersoni* indicated the following:

Development within the host - The rate of establishment (as a percent of the inoculum) was higher in MD than in WTD. Although the development time needed to reach patency was similar in both host species, the worms appeared to disperse

from dorsal muscles to lateral and ventral sites more slowly in MD than in WTD.

Reproductive capacity - There was no apparent difference in the reproductive capacity of *P. andersoni* in WTD and MD. Sex ratios, sex associations, and specific location of adult worms also were similar in both host species. Larval output was variable and overlapping between WTD and MD.

Host defence response - Host response and ability to withstand insult appeared to differ between the two hosts. Clinical signs indicative of respiratory distress were seen in MD but not WTD exposed to 300 larvae. Gross lesions noted in the skeletal muscles, lungs, and lymph nodes were slightly more severe in MD than WTD. The differences appeared to relate to the immunologic response of the hosts (see discussion, Chapter III).

Based on the criteria mentioned earlier, I suggest that WTD were better adapted than MD to infection with *P. andersoni*. The higher establishment rate in MD did not provide higher reproductive output. Instead, it resulted in more damage to the host.

Information from deer exposed to *Parelaphostrongylus odocoilei* can be summarized as follows:

Development within the host - The prepatent period appeared to be longer in WTD than in MD exposed to larvae of *P. odocoilei*. The rate of establishment was extremely high

in MD but appeared low in WTD. Although no adult worms were recovered from WTD, the data collected from fawns exposed to known *P. odocoilei* larvae were consistent for all animals and suggested a few worms reached maturity. As indicated in Chapter III, low numbers of adult worms could easily be overlooked in WTD with the necropsy technique used early in the study.

Reproductive capacity - Larval output was markedly different in MD and WTD. In MD, a pattern of exponential increase to extremely high levels was noted in all animals. Larval output in WTD exposed to *P. odocoilei* was erratic and low level.

Host defence response - The major response to *P. odocoilei* infections was a granulomatous inflammation associated with eggs and larvae. Due to the large number of these stages present in MD, damage associated with the response was extensive and severe. In addition, adult worms were interwoven between muscles and, thus, caused more damage in the final host. The damage resulted in debilitation and loss of condition in MD fawns. Few eggs and larvae were present in WTD and, thus, damage was localized and minor. No clinical signs or loss of condition attributable to *P. odocoilei* was observed in WTD.

There were marked differences between the relationships of *P. odocoilei* in WTD and MD. Using the previous criteria, WTD did not appear to be well-adapted to this parasite. Although there was minimal damage to the host, establishment

rate and larval output were extremely low. In contrast, establishment rate and larval output were high in MD but damage to the host was extensive and severe. These data suggested that MD, also, were not well-adapted to *P. odocoilei*.

It has been suggested that host response may be the major determinant (Wakelin 1976) and pathogenicity the chief characterisitic of the interrelationship between the hosts and the parasite (Dogiel 1964). Pathogenicity becomes particularly important in the relationship if it is directed against the reproductive products. This situation is contrary to the basic premise of a successful host-parasite relationship since maximal reproductive output is not compatible with minimal damage to the host. For example, in *P. odocoilei* infections, tissue damage was associated largely with eggs and larvae. In MD exposed to *P. odocoilei*, the host response to the high numbers of larvae resulted in extensive damage to vital tissues and a noticeable detrimental effect on the host.

The previous conclusions are based on traditional theories of host specificity. By the nature of the relationships, the conclusions are strongly influenced by the level of pathogenicity in each host. However, recently, the premise that a well-adapted relationship exhibits minimal damage to the host has been severely criticized by workers using an ecological approach to specificity. Levine

and Pimentel (1981) and Anderson and May (1982) argue that some pathogenicity in the definitive host is necessary to maintain parasite populations. However, the level of pathogenicity which can be tolerated and still allow maintenance of the parasite population appears to depend on the efficiency of transmission (Anderson and May 1982). Without knowing the details of transmission, it is not possible to determine *a priori* what that level of pathogenicity may be.

Here, I think, one must make the distinction between the theoretical versus the practical value of an evaluation of a host-parasite relationship. Dosage levels used in this study were chosen to allow characterization of the damage by the parasite and the response by the host. Levels also were similar to those used in previous studies and therefore allowed comparison to literature reports. At these levels, MD did not appear to be well-adapted to *P. odocoilei*. However, in field situations, populations of MD in Alberta are known to maintain populations of *P. odocoilei* (Samuel 1978). Thus, theoretically, MD were apparently not well-adapted to *P. odocoilei*, yet, practically, they are suitable definitive hosts.

Do these differences invalidate the traditional methods of evaluating host-parasite relationships? No. A comparison of relationships under controlled experimental conditions allows a comparative evaluation of the adaptations between a

parasite and its hosts. Differences may reflect the extent of coevolution between the organisms. However, such data can not be used in a practical evaluation of host specificity. Such data must come from natural infections in which the associated ecologic factors impinging on transmissibility of the parasite to the host are allowed to act (for example, see Sprent 1963, Holmes et al. 1977).

There is now sufficient information to conduct a wider evaluation of the relationships of *P. odocoilei* with various hosts (Table 1). I shall draw on information detailed in this study, but also use data and inferences from experimental and natural infections reported in the literature (see Hobmaier and Hobmaier 1934, Brunetti 1969, Platt and Samuel 1978) or evaluated in our laboratory. However, the reader is cautioned of the speculative nature of this evaluation.

There is little doubt that coevolution between some parasites and their hosts has occurred (Holmes in press.) but, there are conflicting views about whether adaptation has implications about the age of the relationship in evolutionary terms (see Brooks 1979). Ashlock (1974) concludes that each instance of potential co-evolution must be assessed independently.

The crucial elements in an evaluation of host-parasite relationships appear to be the trade-off between

Table 1. The relationship of Parelaphostrongylus odocoilei and three cervid hosts.

	White-tailed deer	Mule deer	Black-tailed deer
Prepatent period*	65d ?**	50d	50-55d
Establishment	very low	high	low
Location	?	connective tissue of skeletal muscle	connective tissue of skeletal muscle
Larval output	very low	very high	moderate
Lung damage	minor	severe	minor
Muscle damage	none	severe	minor
Overall condition	good-excellent	poor-good	very good

* Following exposure to 300 larvae.

** White-tailed deer may be refractory to infection (see Chapter 2).

reproductive capacity and life span of the parasite (Holmes in press.). [High reproduction is usually associated with high pathogenicity (Anderson and May 1982, and above discussion) which reduces the life span of the host (and, consequently, the parasite)]. Further, Holmes suggests that high reproduction (and, thus, pathogenicity) may be selected for in situations where 1) there is a large susceptible host population and a high rate of transmission (e.g. certain microparasites), 2) death of the host promotes transmission of the parasite (e.g. *Capillaria hepatica* in small rodents), or 3) the life span of the host is short and there is a rapid turnover in the population (e.g. myxosporideans in fish).

Parelaphostrongylus odocoilei and its hosts do not appear to meet these criteria. It is therefore reasonable to assume that direct pathogenicity in the definitive host would not be advantageous to maintenance of this parasite. However, it is possible that high reproductive output (and pathogenicity) may be a necessity for transmission in specific local situations.

Although *P. odocoilei* was capable of establishing infections in various hosts, the relationships differed markedly in their pathogenicity. In experimental infections, pathogenicity was minimal in WTD (Chapter IV), moderate in BTD (Appendix II and pers. observ.), and high in MD (Chapter IV) and moose (Appendix I). Assuming selection for intermediate levels of pathogenicity (see Anderson and May

1982), these observations suggested that BTD may have had the longest association with *P. odocoilei*. In this host, reproductive output appeared sufficient to maintain the parasite population with a reduced interference with the host.

Odocoileus and *Parelaphostrongylus* are both of New World origin (Cowan 1956, Pryadko and Boev 1971, respectively) and it has been suggested that there is strong evidence of coevolution (Pryadko and Boev 1971, Platt 1978). Within the genus *Odocoileus*, *O. virginianus* is generally considered the ancestral form (Geist 1981). Geist (1981) also suggests that the coastal BTD is the ancestral form of *O. hemionus* while Rocky mountain mule deer is the most advanced (and, thus, the most recent). Why, then, should *P. odocoilei* be adapted to the ancestral form and not the derived form?

Black-tailed deer ranged throughout western North America prior to the ice ages (Cowan 1956). Subsequently, much of this range was lost. However, the coastal region of the Pacific Northwest was largely unaffected by the ice age (Waring and Franklin 1979). The long period of stability in coastal BTD may have allowed continued adaptation between the host and parasite resulting in a well-adapted relationship.

Mule deer apparently arose rapidly [from ancestral BTD] in mountain refugia during the last ice age (Cowan 1956). Parasite evolution often lags that of the host (Kennedy

1975) and MD may have been unable to maintain a population in the new host and/or habitat. As the ice retreated, this new race expanded its range and became the numerically and geographically dominant subspecies. In so doing, they again overlapped in distribution with the ancestral black tail form.

The new subspecies (MD), with its different physiology and behaviour, would also be exposed to *P.odocoilei*. The host-parasite relationship of *P. odocoilei* in MD shows the characteristics of a relatively recent association which has not reached the balance between promoting and inhibiting factors. Reproductive output is sufficient to maintain the parasite population; however, there is considerable interference with the host.

In contrast to the recent exposure of MD to *Parelaphostrongylus*, WTD appear to have had a long association with this nematode genus. The adaptations and stability of the relationship between WTD and *P. tenuis* are readily apparent (see Anderson 1971). Limited establishment rates and moderate host response in WTD also suggest a well-balanced relationship (and long association) with *P. andersoni*. This 'pre-adaptation' to *Parelaphostrongylus* spp. may act more strongly on *P. odocoilei* and result in an exaggerated limitation of the infection in WTD.

Moose originated in Eurasia and crossed the Bering land bridge into North America (Flerov 1960, Kurten 1968). Thus, they have had a limited association with species of

Parelaphostrongylus. Both *P. tenuis* and *P. odocoilei* are able to establish infections in moose; however, pathogenicity is high in such infections (Anderson 1964, Appendix I). Moose are obviously not well-adapted to *P. odocoilei*.

Although, the current data allow a descriptive evaluation of the host-parasite relationships, the underlying mechanism affecting the balance between reproduction and pathogenicity remains unknown. Why did 20% of the *P. andersoni* larvae mature in WTD yet 50% of the *P. odocoilei* larvae did so in MD? Where and why were the remaining larvae lost from the system?

Parasites within the tissues and cells of the host are in extremely close physiological union with their host (Jones 1967). Neither species of *Parelaphostrongylus* was able to mature in the rodent and lagomorph hosts examined in this study (see Appendix III) and it appeared that the worms may develop only in a ruminant. However, *P. odocoilei* did not establish infections in domestic goats (Appendix III) and *P. andersoni* was absent in feral cattle and swine sympatric with populations of WTD infected with *P. andersoni* (Prestwood et al. 1975). Thus, the resource may be specific to cervids and wild bovids.

Differences in establishment rates appear to relate to the development of the infective larvae. However, activation of nematode third-stage larvae is apparently governed by

non-specific factors found in a variety of hosts (Rogers 1966) and probably plays a minor role in determining specificity (Kennedy 1975). Thus, the differences may involve penetration, migration, and maturation of larvae within the definitive host. These aspects of *P. andersoni* and *P. odocoilei* infections have not been investigated.

In conclusion, the current program uncovered the need for further investigation of these host-parasite relationships under both natural and experimental conditions. Field investigation of host range, transmission rates, exposure levels, and establishment rates are necessary to complete the study of host specificity. Evaluation of experimental infections starting immediately after exposure to infective larvae could provide information about how the specificity is mediated.

LITERATURE CITED

Anderson, R.C. 1964. Neurologic disease in moose infected experimentally with *Pneumostrongylus tenuis* from white-tailed deer. Path. Vet. 1: 289-322.

Anderson, R.C. 1971. Lungworms. *In*: Parasitic diseases of wild mammals. J.W. Davis and R.C. Anderson (eds.). The Iowa State Univ. Press, Ames, Iowa. pp 81-126.

Anderson. R.M., and R.M. May. 1982. Coevolution of hosts and parasites. Parasitol. 85: 411-426.

Ashlock, P.D. 1974. The uses of cladistics. Ann. Rev. Ecol. System 5: 81-99.

Barbehenn, K.R. 1969. Host-parasite relationships and species diversity in mammals: an hypothesis. Biotropica 1: 29-35.

Brooks, D.R. 1979. Testing the context and extent of host-parasite coevolution. Syst. Zool. 28: 299-307.

Brunetti, O.A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game 55: 307-316.

Cowan, I. McT. 1956. What and where are the black-tailed deer? *In*: The deer of North America. W.P. Taylor (ed.), The Stackpole Co., Harrisburg, Penn. and The Wildlife Manage. Inst., Wash., D.C. pp 335-359.

Dogiel, V.A. 1964. General Parasitology. Oliver and Boyd, Edinburgh and London. 516pp. [English translation by Z. Kabata].

Flerov, K.K. 1960. Fauna of USSR mammals. Vol 1: Musk deer and deer. [English translation IPST Cat. No. 123].

Garber, E.D. 1956. A nutrition-inhibition hypothesis of pathogenicity. Am. Natural. 90: 183-194.

Geist, V. 1981. Behavior: adaptive stategies in mule deer. *In*: Mule and black-tailed deer of North America. O. C. Wallmo (ed.), Univ. Nebraska Press, Lincoln. pp 157-223.

Hobmaier, A., and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n. sp., a new lungworm in black tail deer (*Odocoileus columbianus*). Description and life history. Proc. Soc. Exp. Biol. Med. 31: 509-514.

Holmes, J.C. In press. Evolutionary relationships between parasitic helminths and their hosts. *In*: Coevolution. D.J. Futuyma, M. Slatkin, J. Roughgarden, and B. Levin (eds.), Sinauer Associates, Sunderland, Mass. 82pp.

Holmes, J.C., R.P. Hobbs, and T.S. Leong. 1977. Populations in perspective: community organization and regulation of parasite populations. *In*: Regulation of parasite populations. G.W. Esch (ed.), Academic Press, New York. pp 209-245.

Jones, A.W. 1967. Introduction to parasitology. Addison-Wesley Publ. Co., Reading. 458pp.

Kennedy, C.R. 1975. Ecological Animal Parasitology. Blackwell Scientific Publ. Oxford. 163pp.

Kurten,B. 1968. Pleistocene mammals of Europe. Aldine Publ. Co., Chicago. 317pp.

Levin, S., and D. Pimentel. 1981. Selection of intermediate rates of increase in parasite-host systems. Am. Natural.

117: 308-315.

Lewis, R.W. 1953. An outline of the balance hypothesis of parasitism. Am. Natural. 87: 273-281.

Odening, K. 1976. Conception and terminology of hosts in parasitology. Adv. in Parasitol. 14: 1-93.

Platt, T.R. 1978. The life cycle and systematics of *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea), a parasite of mule deer (*Odocoileus hemionus hemionus*), with special reference to the molluscan intermediate host. Ph.D. Thesis, University of Alberta, Edmonton, Alberta. 233pp.

Platt, T.R., and W.M. Samuel. 1978. *Parelaphostrongylus odocoilei*: life cycle in experimentally infected cervids including the mule deer, *Odocoileus h. hemionus*. Exp. Parasitol. 46: 330-338.

Prestwood, A.K., F.E. Kellogg, S.R. Pursglove, and F.A. Hayes. 1975. Helminth parasitisms among intermingling insular populations of white-tailed deer, feral cattle, and feral swine. J. Am. Vet. Med. Assoc. 166: 787-789.

Pryadko, E.I. and S.N. Boev. 1971. Systematics, phylogeny, and evolution of elaphostrongyloid nematodes of deer. Izdatel. Akad. Nauk. Kaz. 5: 41-48.[In russian]

Rogers, W.P. 1966. Exsheathment and hatching mechanisms in helminths. *In*: Biology of parasites. Academic Press Inc., New York. pp 33-40.

Samuel, W.M. 1978. Parasite research in national parks of western Canada. II. Muscleworm of mule deer. Unpub. rpt. for Parks Canada. pp 84-143.

Sprent,J.F.A. 1963. The life history and development of *Amplicaecum robertsi*, an ascaridoid nematode of the carpet python (*Morelia spilotes variegatus*). Parasitol. 53: 321-337.

Wakelin, D. 1976. Host responses. *In*: Ecological aspects of parasitology. C.R. Kennedy (ed.) pp 115-141.

-----. 1978. Genetic control of susceptibility and resistance to parasitic infection. Adv. Parasitol. 16: 219-308.

Waring, R.H. and J.F. Franklin. 1979. Evergreen coniferous forests of the Pacific Northwest. Science 204: 1380-1386.

VII. Appendix I: Pathology of the muscleworm, *P. odocoilei* (Nematoda: Metastrongyloidea), in moose.

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ABSTRACT

Parelaphostrongylus odocoilei is a muscleworm common in mule deer, *Odocoileus hemionus hemionus*, of western Alberta. Its effects in alternate hosts is essentially unknown. Two moose calves, *Alces alces*, were each given either 300 or 800 third-stage larvae of *P. odocoilei*. At necropsy, particular attention was given to gross lesions and samples for histopathologic study. Gross lesions in moose consisted of myositis in the back and hind quarters accompanied by general softening of tissue, lymph node hypertrophy and petechial haemorrhage throughout the lungs. Histopathologic examination confirmed chronic progressive myositis in skeletal muscles and increased activity of the lymphoid system. Atelectasis, interstitial pneumonia, and interlobular oedema were common in lung sections. Severity of infection in moose relative to that in mule deer is discussed briefly.

INTRODUCTION

Differential host susceptibility or host specificity is a common feature of parasitic infections (Holmes 1976). In 1964, Anderson showed that moose (*Alces alces*) of North America are particularly susceptible to insult as a result of infection with meningeal worm, *P. tenuis*. Adult worms enter the central nervous system and cause extensive damage to nervous tissue. White-tailed deer (*Odocoileus virginianus*) can apparently harbour the worm with little effect (Anderson 1963).

Other members of the genus *Parelaphostrongylus* are also present within the normal range of moose yet their effect on moose is unknown. Recently, Platt and Samuel (1978) reported a muscleworm, *P. odocoilei*, in mule deer (*O. hemionus hemionus*) of western Alberta. Pybus and Samuel (1981) report *P. andersoni*, also a muscleworm, in white-tailed deer of southeastern British Columbia. Since moose share ranges with mule deer in western Alberta (pers. observ.) and white-tailed deer in southeastern B.C. (G. Tipper, pers. comm.), the potential exists for exposure of moose to muscleworms. The following study was undertaken to determine pathological effects of *P. odocoilei* on moose calves.. Susceptibility and severity of infection in moose and mule deer are compared briefly.

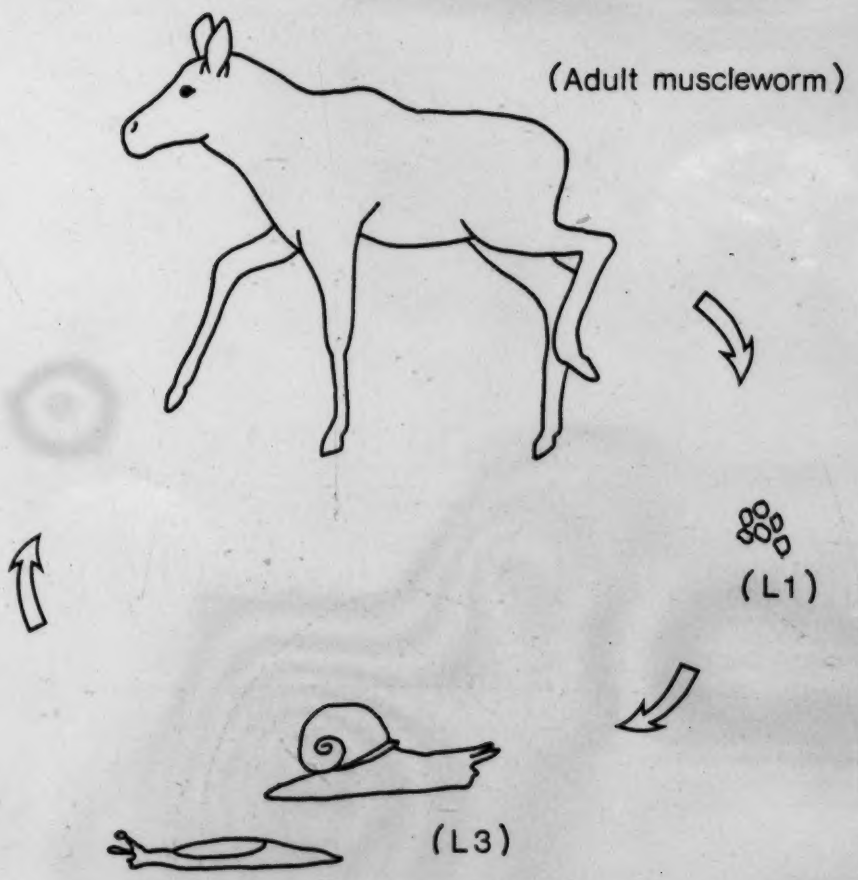
MATERIALS AND METHODS

In 1978, 2 moose approximately 4-5 weeks old were received from the Fish and Wildlife Division of the Alberta Department of Energy and Natural Resources. Animals were initially bottle-fed a 2:1 mixture of whole milk and evaporated milk for the first 2 weeks of captivity and then gradually switched to milk replacer. Two to 3 weeks after arrival, animals were given access to a grassy paddock which had not previously contained animals infected with *P. odocoilei*. Calves were weaned to a diet of commercial deer pellets, beet pulp and hay at approximately 3 1/2 months-of-age.

First-stage larvae (L1's) of *P. odocoilei* were collected from faecal pellets of infected mule deer by the baermann technique (see Fig. 1 for life cycle). Laboratory-reared snails (*Triodopsis multilineata*) were exposed to these larvae. After a minimum of 30 days, snails were digested in pepsin-HCl digest (0.6 g pepsin in 0.7 ml HCl per 100 ml distilled water). Third-stage larvae (L3's) were collected and administered *per os* to calves. One moose (Mo 12) received 300 L3's while the remaining moose (Mo 10) received 800 L3's. Daily faecal samples were collected and examined for L1's from 40 to 95 and 40 to 98 days post exposure (dPE) for Mo 12 and Mo 10, respectively. Weekly faecal samples were collected and monitored thereafter until 101 dPE (Mo 12) and 154 dPE (Mo 10).

Figure 1. Life cycle of Parelaphostrongylus odocoilei in moose.

(L1 = first-stage larva, L3 = third-stage larva)


(Adult muscleworm)
(L1)
(L3)

Animals were terminated with sodium pentabarbitol injected into the jugular vein. Standard necropsy procedures were used to examine all carcasses. Samples from various organs and tissues were collected and fixed in 10% neutral buffered formalin for histopathologic examination. In addition, skeletal muscles were removed individually. Longissimus dorsi and psoas muscles were dissected at 6X magnification for recovery of worms. Muscles of the hind quarters and abdominal wall were sliced thinly (3-5mm) and searched for haemorrhages. All haemorrhagic areas were examined at 6X magnification for presence of adult worms. Muscles of the fore quarters were thick-sliced (8-10 mm) and treated as those of the hind quarters. Muscleworms recovered were fixed in hot glycerin alcohol and cleared for examination to glycerin. Tissue samples of haemorrhagic and/or necrotic areas as well as worms *in situ* were fixed in formalin. Approximately 200 g of lung tissue from Mo 10 was cubed and left overnight in pepsin-HCl digest at 37°C.

Histological preparations were sectioned at 7 micrometers, stained in haematoxylin and eosin and examined for pathologic lesions. Size and number of granulomas and per cent consolidatation of lung tissue were determined in each of 10 sites throughout the lungs. Lesions were described with the assistance of Dr. R.J. Lewis, Animal Health Division, Alberta Department of Agriculture.

RESULTS

Both moose developed patent infections of *P. odocoilei* (Table 1). No animal showed clinical signs attributable to infection with muscleworm; that is, abnormal stance, movement, respiration, or response to touch.

Gross Necropsy

At 101 dPE, 6 adult *P. odocoilei* were recovered from the left psoas and left biceps femoris of Mo 12. Worms were associated with localized haemorrhage and usually found in pairs (1male, 1female). Haemorrhagic tracts and focal haemorrhage were noted throughout various muscles but often worms were not located in the vicinity. Extensive haemorrhage and focal necrosis were noted throughout skeletal muscles of the back, fore, and hind quarters of Mo 10. Muscles were soft and pale and fibres separated easily. Haemorrhagic areas varied from 5-15 mm in diam. Necrotic areas varied from small localized areas (1 mm diam.) to much larger areas (10-15 mm diam.). Yellow viscid exudate and/or green caseous material was present in the large areas. Approximately 1/2 of the necrotic areas were associated with haemorrhage and these areas often extended up to 20-30 mm in diam. and 80-90 mm in length. Twenty-three adult *P. odocoilei* were recovered from the psoas, longissimus dorsi, and various muscles of the thigh of Mo 10. Worms were usually associated with haemorrhage but never with necrotic areas. As in Mo 12, haemorrhage was often found in the

Table 1. Infection data for Parelaphostrongylus odocoilei in moose.

	Dose	Prepatent period (days)	dPE*	Maximum weekly larval output**	Worm recovery***
Moose 10	800	70	217	108.5 (wk. 9)	23 (2.9)
Moose 12	300	67	101	0.2 (wk. 2)	6 (2.0)

* Days post exposure.

** Larvae per gram wet weight faeces.

*** Number of worms (per cent of dose).

absence of worms. Tissue degeneration involved approximately 10-20% of an infected muscle. Petechial haemorrhage was present throughout all lobes of the lungs of both moose. Diaphragmatic lobes were slightly darkened but of normal consistency. Larval recovery from the lungs of Mo 10 was 14.4 larvae/gram.

Histopathology

Adult *Parelaphostrongylus odocoilei* were found in the perimysium of infected muscles. Worms were occasionally adjacent to blood vascular tracts in the perimysium and the posterior portion of 1 female worm was oriented parallel to a vein and artery. Worms were rarely found between the fibres within a muscle bundle. Adult worms in the perimysium were usually surrounded by a loose connective tissue 'coat' with a clear open space between the worm and the connective tissue. This finding was consistent for all worms but could be an artifact of preparation.

Adult worms elicited a consistent response only when found within a muscle bundle. In such cases, a minor buildup of polymorphonuclear cells (neutrophils) and mononuclear cells (macrophages) was seen. Worms in the perimysium rarely were associated with a cellular response.

Large numbers of nematode eggs were located in perimysium as well as within muscle bundles of the back and thighs. Eggs were found in groups or clumps regardless of the site. Clumps of eggs were never found directly

associated with a female worm. Occasionally, 'trails' of eggs were found through a number of muscle fibres and adjacent muscle bundles. Within each group, eggs were usually at a similar stage of development. Most were in the 2-8 cell stage of cellular division; seldom were they beyond the 16 or 32 cell stage.

The presence of eggs in the perimysium or between muscle fibers inevitably resulted in a massive cellular buildup in the area. This response appeared to start in the perimysium and then spread into adjacent muscle bundles. Most of the infiltration was concentrated directly around the eggs. Eggs were often seen enmeshed in a loose fibrous matrix that also contained invading white blood cells. There appeared to be progressive stages in response to the insult and lesions were indicative of chronic progressive myositis. Lesions were similar regardless of muscle involved.

Groups of eggs were extremely large in most cases and the associated cellular buildup often destroyed large (100-200 mm) areas of muscle. The basic organization of the muscle was destroyed throughout large tracts; that is, there were no longer discrete muscle bundles separated by narrow perimysium. Little evidence of repair was noted, even in obviously chronic lesions.

Very few first-stage larvae of *P. odocoilei* were found in muscle preparations. When present, they were coiled in loose connective tissue within muscle bundles and surrounded by a loose connective tissue capsule. Within the capsule,

numerous giant cells and epithelial cells were attacking the coiled larva. No evidence of degeneration was noted in the larvae. A diffuse polymorph and mononuclear buildup was seen in surrounding muscle tissue.

Localized focal granulomas in various progressive stages were present throughout all lobes of the lungs. In each animal, size of granulomas and number of granulomas per mm^2 were similar regardless of lobe or side of the lung (left vs. right). Size of granulomas was also similar between the 2 animals. However, number of granulomas per mm^2 was significantly greater in Mo 10 ($\bar{x}$=0.25) than in Mo 12 ($\bar{x}$=0.05) (t=5.65, d.f.=18). There was slightly more consolidation in cardiac lobes than in diaphragmatic lobes in both animals.

Focal atelectasis and vasculitis were the most prominent lesions in the lungs of Mo 12. Severe arteritis was noted around a large collapsed bronchial artery in the left cardiac lobe. Collapse and/or occlusion of small arteries was also common in this lobe. Interlobular oedema was present throughout all lobes.

Interstitial pneumonia was evident in Mo 10 as an increase in smooth muscle tissue and thickening of alveolar walls. Response was often centered around focal granulomas containing nematode eggs and larvae. Intensity of response decreased with increasing distance from the granulomas. Lymphoid tissue was active in all lobes. Atelectasis and vascular collapse were minor insults in this animal. Many

bronchioles contained mucous exudate with red and white blood cells present. Lymphoid stasis and venous thrombus were occasionally observed.

Most eggs and larvae observed in the lungs appeared viable. All were associated with a marked cellular response and none was found free in alveolar spaces.

Inguinal, mesenteric, and axillary lymph nodes in both moose were hypertrophic with moderately to heavily active centers. Eggs and larvae of *P. odocoilei* were found in clumps in the subcapsular spaces and medulla of axillary lymph nodes of Mo 10. The subcapsular space was occluded by a granulomatous response characterized by lymphocytes and macrophages. Germinal centers adjacent to eggs and larvae were very active and capped with lymphocytes. Peripheral afferent lymphatics were occluded with eggs and larvae and light pink-staining fluid indicative of oedema was present throughout the medulla of the node. Some eggs had apparently been overcome and subsequently invaded by mononuclear cells. An extensive buildup of mononuclear cells was seen around all free larvae. A few nematode eggs were also found in an axillary lymph node of Mo 12. Response was similar to that described above. Rumenal lymph nodes were inactive in both animals.

The spleen was moderately active in Mo 10 but relatively inactive in Mo 12. Germinal centers were similar in size in both animals. The spleen in Mo 10 was enlarged and congested with red blood cells, that of Mo 12 was

greatly contracted. Neutrophils and haemosiderin were common in spleen samples of both moose.

Within the liver of both animals, extensive oedema and distension of lymphatics was noted in portal triads. Minor mononuclear cellular infiltration was also present in these areas.

Tissue samples were taken from each of the greater and lesser curvature of the fundic region and the greater and lesser curvature of the pyloric region of each abomasum. A first-stage larva was found within the mucosa of the sample from the greater curvature of the pyloric region of Mo 10. A tract of mononuclear cells was present leading from the epithelial border towards the muscularis mucosa. A large accumulation of mononuclears and polymorphs was present anterior to the larva and normal mucosal morphology was destroyed in this area. Lesions were not seen in the abomasum of Mo 12.

To summarize the histopathologic results, it is apparent that eggs and larvae provide the major antigenic stimuli eliciting a marked white blood cell response. Response is concentrated around eggs and larvae but is diffuse throughout large areas of both skeletal muscle and lung tissue. Extensive degeneration and necrosis of muscle fibres occurs in a chronic progressive myositis. The response starts in the perimysium and spreads into adjacent muscle bundles. As a result, tissue degeneration is not localized but extends throughout infected muscles.

Polymorphs and mononuclears attempt to remove debris from haemorrhage and necrotic areas in muscles. Large giant cells are found adjacent to eggs and larvae. Insult appears to be more severe in cardiac lobes and at higher dosage levels.

DISCUSSION

Large numbers of eggs were seen in the perimysium and within muscle bundles yet few female worms were present. Most eggs were in early developmental stages, even in chronic lesions. Few first-stage larvae were seen. It appears that eggs are indiscriminantly released as females move through muscle bundles. Development of eggs may be inhibited or delayed in muscle tissue. It is possible that eggs may require oxygen for development (a common requirement in nematodes (Chitwood and Chitwood 1950)) and cannot get sufficient amounts in skeletal muscle. This would suggest much of the reproductive potential of females is lost as eggs are trapped in the muscles.

Eggs apparently provide a persistent antigenic stimulus which is difficult to overcome and elicits a tremendous cellular response. The result is extensive necrosis and prevention of repair. This would account for the large areas of caseous necrosis and weak muscle tissue noted at necropsy of Mo 10. Similar gross lesions in the skeletal muscle of 1 moose experimentally infected with *P. odocoilei* were noted by Platt (1978). Nettles and Prestwood (1976) described similar lesions in white-tailed deer infected with *P.*

andersoni. Such lesions were seen only in heavily infected animals receiving 1000 or 5000 larvae. White-tailed deer exposed to lower levels of *P. andersoni* (5 to 356 L3's) did not show extensive lesions (Nettles and Prestwood 1976, Prestwood and Nettles 1977, Chapter IV)

Prepatent periods in the present study were similar to those reported for other moose exposed to *P. odocoilei* (Platt and Samuel 1978). Number of larvae per gram of faeces was similar at lower doses but much greater at higher doses than those previously reported by Platt and Samuel (1978). In comparison to mule deer given similar numbers of larvae, prepatent period is longer and larval output is much lower in moose (Platt and Samuel 1978, Chapter III).

Nettles and Prestwood (1976) reported abnormal stance and gait, decreased physical strength, and reluctance to stand in 2 white-tailed deer exposed to 5000 larvae of *P. andersoni*. The absence of such signs in moose infected with *P. odocoilei* (Platt and Samuel 1978, present study) may indicate that muscle lesions are of little consequence to the animal. However, relatively severe damage was noted in a number of muscles of the back and thighs. Under sustained use or in critical situations faced by free-ranging animals, such damage could have a negative effect on fitness. In addition, duration of egg production by female worms and persistence of eggs and larvae in muscles are unknown. Histologic preparations examined in this study indicate that muscle repair is slow in the presence of eggs and larvae. In

combination, these factors could put the host at a disadvantage.

Considering the reserve capacity of the lungs, it would appear that the insult to the lung would be of minor importance in the overall health of an infected animal.

Necrosis and destruction of muscle tissue is much greater in moose than in mule deer exposed at similar levels of *P. odocoilei* (see Chapter V). However, lesions in the lungs are less severe in moose. This may be related to the percentage of worms which are able to establish in skeletal muscle and subsequently contribute to egg and larval production. Two to 3% of the initial dose was recovered as adult worms in moose. This compares to 45% recovery from mule deer. Lower production of eggs, accompanied by loss of eggs and larvae trapped or destroyed in the muscles, results in much less insult to the lungs of moose. Thus, moose cannot be considered more susceptible to *P. odocoilei* than mule deer even though worms which successfully mature cause relatively severe damage in skeletal muscles.

There appears to be widespread activation of the lymphoid tissue in moose infected with *P. odocoilei*. This may be an indication of the strength and/or amount of antigenic stimulus provided by eggs and larvae. Unfortunately, similar histopathologic studies have not yet been completed for *P. odocoilei* in deer. Regardless of whether this response is higher or lower than that in deer, such a challenge to the immune system could become an

important factor in determining how a free-ranging animal responds to additional stresses and insults.

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LITERATURE CITED

Anderson, R.C. 1963. The incidence, development, and experimental transmission of *Pneumostrongylus tenuis* Dougherty (Metastrongyloidea: Protostrongylidae) of the meninges of the white-tailed deer (*Odocoileus virginianus borealis*) in Ontario. Can. J. Zool. 41: 775-792.

-----, 1964. Neurologic disease in moose infected experimentally with *Pneumostrongylus tenuis* from white-tailed deer. Pathologia Vet. 1: 289-322.

Chitwood, B.G. and M.B. Chitwood. 1950. Introduction to Nematology. Univ. Park Press, Baltimore. 334pp.

Holmes, J.C. 1976. Host selection and its consequences. *In*: Ecological aspects of parasitology, C.R. Kennedy (ed.). North-Holland Publ. Co., Amsterdam. pp 21-39.

Nettles, V.F. and A.K. Prestwood. 1976. Experimental *Parelaphostrongylus andersoni* infections in white-tailed deer. Vet. Pathol. 13: 381-393.

Platt, T.R. 1978. The life cycle and systematics of *P.*

odocoilei (Nematoda: Metastrongyloidea), a parasite of mule deer (*Odocoileus hemionus hemionus*), with special reference to the molluscan intermediate host. Ph.D. Thesis, Univ. Alberta, Edmonton. 233pp.

Platt, T.R. and W.M. Samuel. 1978. *P. odocoilei*: life cycle in experimentally infected cervids including mule deer, *Odocoileus h. hemionus*. Exper. Parasitol. 46: 330-338.

Prestwood, A.K. and V.F. Nettles. 1977. Repeated low-level infection of white-tailed deer with *Parelaphostrongylus andersoni*. J. Parasitol. 63: 974-978.

Pybus, M.J. and W.M. Samuel. 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. 45: 537-542.

VIII. Appendix II: Natural Infections of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in several Hosts and Locations.

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ABSTRACT

Parelaphostrongylus odocoilei is reported from black-tailed deer (*Odocoileus hemionus columbianus*) on Vancouver Island, British Columbia, a white-tailed deer/mule deer hybrid from Alberta, and two mountain goat (*Oreamnos americanus*) from Washington and Alberta. This is the first report of *P. odocoilei* in a non-cervid definitive host. These findings, along with other published information, indicate that *P.odocoilei* may have a broad distribution in northwestern North America.

INTRODUCTION

Parelaphostrongylus odocoilei (Hobmaier and Hobmaier 1934) is a nematode parasite common in the skeletal muscles of mule deer (*Odocoileus hemionus hemionus*) in Alberta (Samuel 1978). It is reported also from black-tailed deer (*O. h. columbianus*)(BTD) and California mule deer (*O. h. californicus*) in California (Hobmaier and Hobmaier 1934, Brunetti 1969). Experimental infections have been reported in moose (*Alces alces*) (Platt and Samuel 1978, Pybus and Samuel 1980). Platt and Samuel (1978) report a lack of patent infections in seven white-tailed deer (*O. virginianus*) experimentally exposed to *P. odocoilei*; however, seven white-tailed deer exposed to larvae of *P. odocoilei* in the current study apparently became infected (Chapter III).

Parelaphostrongylus odocoilei is a pathogen of individual mule deer (Brunetti 1969) and has the potential to be an indirect mortality factor in mule deer populations (Chapter V). It can cause extensive damage in moose (Pybus and Samuel 1980).

First-stage larvae indistinguishable from those of *P. odocoilei* (and a few other closely-related nematodes) have been collected from the faeces of a variety of cervid and bovid populations in western Canada (Pybus and Samuel 1981 and our unpubl. data). Recently, we attempted to collect and identify adult worms from hosts passing these dorsal-spined first-stage larvae.

In this paper we report the recovery of *P. odocoilei* from two hosts and two geographic locations not previously known. The results have important bearing on the distribution and host specificity of this parasite.

MATERIALS AND METHODS

Black-tailed deer - In June, 1977, 16 faecal samples were collected from BTD habitat on Vancouver Island, British Columbia (50°N, 126°W). Samples were examined using the Baermann technique (as in Platt and Samuel 1978). Two *Triodopsis multilineata* were exposed to larvae collected from the Baermann funnels. Thirty seven days later, the snails were digested in pepsin-HCl at 37°C and 300 larvae recovered were administered to a nine-week old BTD raised at the University of Alberta Biomedical Animal Centre Ellerslie, Alberta without access to snails. The deer was killed 262 days later and a routine necropsy conducted. In addition, the backstrap muscles (longissimus dorsi, psoas, and iliacus) were shredded and examined at 6X magnification. All other skeletal muscles except those on the head were sliced at 5mm intervals and examined for adult helminths. Gross lesions in the muscles were removed and fixed in 10% buffered formalin. All worms recovered were fixed in hot glycerin-alcohol and cleared and examined in glycerin.

Hybrid - In 1980, seven faecal samples collected from September to December from a yearling hybrid female white-tailed deer/mule deer in Jasper National Park (JNP),

Alberta (53°N, 118°W) were examined by the Baermann technique. The deer was captured and transported to the research centre. Daily faecal samples were collected until the doe was killed. The carcass was examined as described above for the BTD.

Mountain goat - In February 1982, an eight-year old male mountain goat (*Oreamnos americanus*) was found near Newhalem, Washington (48°N, 121°W). The animal died and was sent to the Washington State University. At necropsy, areas of gross haemorrhage in the skeletal muscles and lungs were removed and fixed in 10% buffered formalin. Additional skeletal lesions were fixed in formalin and sent to the University of Alberta.

In June 1982, a seven year old female mountain goat was killed on the highway in JNP, Alberta and then transported to Edmonton, Alberta for necropsy. A faecal sample was examined by the Baermann technique. The necropsy methods were as described above for the BTD.

RESULTS

Black-tailed deer - Nine of sixteen (56%) faecal samples collected on Vancouver Island contained dorsal-spined larvae. Intensities were consistently low and ranged from <0.1 to 2.0 larvae per gram wet weight feces (l/g) ($\bar{x}$=0.7+0.6).

A total of twenty nine adult *P. odocoilei* were recovered from the experimentally-infected fawn. (twenty

seven from the skeletal muscles of the back and thighs and two in the gastrocnemius and latissimus dorsi muscles). All worms were associated with focal haemorrhage and localized tissue damage.

Hybrid - This deer had morphologic features indicative of a hybrid white-tailed/mule deer (see Wishart 1980). Gel electrophoresis of serum albumen also indicated a hybrid condition (see McClymont et al. 1982).

All faecal samples collected from this deer contained dorsal-spined larvae. Intensity ranged from 75 to 171 l/g ($\overline{x}$=129). At necropsy, three adult *P. odocoilei* were recovered from the right vastus muscles. They were not associated with any noticeable tissue damage.

Mountain goat- The Washington goat was found in a weakened condition and soon died. It was thin and emaciated with no body fat. Gross lesions included extensive haemorrhage throughout the skeletal muscles and approximately 65 *P. odocoilei* were collected from the muscles. Dorsal-spined larvae were found in the faecal sample taken during the necropsy. A few *Protostrongylus rushi* were recovered from the bronchioles.

Histologic examination revealed focal muscular haemorrhage. Adult nematodes were not associated with any cellular response. Lesions in lung sections were indicative of verminous pneumonia.

The Alberta goat was in excellent body condition with extensive fat deposits throughout the carcass. Six adult *P.*

odocoilei were found in the backstrap muscles. Worms were coiled in pairs (1 male, 1 female) in connective tissue between the muscle bundles. Local haemorrhage (5-15mm diameter) was associated with each pair of worms. Dorsal-spined larvae and larvae of *Protostrongylus* spp. (2.8 l/g) were present in the faeces in a ratio of 2:1.

Gross and histologic results were indicative of a mild verminous pneumonia. The bronchioles did not contain adult helminths; however, *P. stilesi* was recovered from the lung parenchyma. Histologic sections also revealed granulomatous inflammation around dorsal-spined larvae scattered throughout all lobes.

Specimens of *P. odocoilei* from each host have been deposited in the National Museum of Natural Sciences, Ottawa, Ontario accession # XXXX. Table 1 presents selected measurements of specimens.

DISCUSSION

Parelaphostrongylus odocoilei has previously been reported only from cervid definitive hosts (Hobmaier and Hobmaier 1934, Brunetti 1969, Platt and Samuel 1978, Pybus and Samuel 1980). The establishment of a natural infection in a bovid provides evidence that the host specificity of this worm may not be as narrow as previously considered. In addition to animals described herein, adult nematodes have been seen in the skeletal muscles of two mountain goats in Washington (pers. observ.). Dorsal-spined larvae have been

Table 1. Selected measurements (um) of adult Parelaphostrongylus odocoilei from various hosts.

	Mountain goat			Black-tailed
	Washington	Alberta	Hybrid	deer
Male				
N	5	3	1	4
Total length(mm)	39.0*	-	26.2	23.5,23.7**
Left spicule				
total length(A)	156 ± 14	150 ± 0	138	155 ± 4
length split(B)	42 ± 7	34 ± 5	35	40 ± 2
(A/B)100	27 ± 3	23 ± 4	25	25 ± 1
Right spicule				
total length(A)	159 ± 9	148 ± 8	145	151 ± 5
length split(B)	45 ± 4	32 ± 7	36	40 ± 2
(A/B)100	28 ± 3	22 ± 3	25	26 ± 2
Female				
N	3	0	1	4
Total length(mm)	41.4 ± 5	-	43.4	45.1 ± 6

* One complete specimen only.

**Two complete specimens.

observed in 184 of 336 (55%) fecal samples of mountain goats in Alberta, central British Columbia, and Washington (our unpubl. data). Further investigation is necessary to determine whether these goat populations harbour infections of *P. odocoilei*.

The presence of *P. odocoilei* in Vancouver Island (Platt pers. commun., present study) and Washington suggests a broader distribution than was known. Rather than being localized in disjoint populations, *P. odocoilei* may be distributed throughout northwestern North America, perhaps, in a variety of hosts.

These findings have important implications for wildlife managers and wildlife parasitologists throughout northwestern North America. The goat harbouring a natural infection of 65 worms was weak and emaciated. Although there was extensive haemorrhage throughout the muscles, it is unknown to what extent the parasite may have contributed to this condition. Due to its potential as a direct or indirect mortality factor, *P. odocoilei* should be considered when examining big game animals in this region. The findings also provide important information concerning host specificity of *Parelaphostrongylus* spp.

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Fish and Wildlife) conducted the electrophoretic study. Dr. C.W. Leathers (Washington State University) evaluated the histopathology of the Washington goat.

LITERATURE CITED

Brunetti, O. A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game 55: 307-316.

Hobmaier, A., and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n. sp., a new lungworm in black tail deer (*Odocoileus columbianus*). Description and life history. Proc. Soc. Exp. Biol. Med. 31: 509-514.

McClymont, R.A., M. Fenton, and J.R. Thompson. 1982. Identification of cervid tissues and hybridization by serum albumen. J. Wildl. Manage. 46: 540-544.

Platt, T. R. and W. M. Samuel. 1978. *Parelaphostrongylus odocoilei*: life cycle in experimentally infected cervids including the mule deer, *Odocoileus h. hemionus*. Exper. Parasitol. 46: 330-338.

Pybus, M.J. and W.M. Samuel. 1980. Pathology of the muscleworm, *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea), in moose. Proc. N. Am. Moose Conf. and Workshop 16: 152-170.

Pybus, M. J., and W. M. Samuel. 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. 45: 537-542.

Samuel, W.M. 1978. Parasite research in national parks of western Canada. II. Muscleworm of mule deer. Unpubl. rpt. to Parks Canada. pp.84-143.

Wishart, W. 1980. Hybrids of white-tailed and mule deer in Alberta. J. Mammal. 61: 716-720.

IX. Appendix III: Attempts to find a laboratory host for *Parelaphostrongylus andersoni* and *P. odocoilei* (Nematoda: Protostrongylidae).

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ABSTRACT

Twenty guinea pigs (*Cavia porcellus*) and eight domestic rabbits (*Oryctolagus cuniculus*) were each exposed to 100 or 200 third-stage larvae of *Parelaphostrongylus andersoni* or *P. odocoilei*. A few larvae successfully penetrated the stomach and caecal walls and were later found in the mesenteries, liver, and diaphragm. However, the majority of larvae were recovered from the lungs, and connective tissues of the pleural cavity. Growth and development of the third-stage larvae were not apparent and larvae did not reach the skeletal muscles, the normal definitive site for *P. andersoni* and *P. odocoilei*. All larvae in tissues were encapsulated (after 36 days) and overcome (after 59 days) by a local host response. Larvae appeared to migrate via the circulatory system and by direct penetration of tissues and organs.

Three domestic goats (*Capra hircus*) failed to pass larvae of *P. odocoilei* in the faeces for up to 101 days after exposure to 300 or 1000 third-stage larvae. Thus, none of the hosts examined in this study appear suitable as an alternative to the cervid final hosts for *P. andersoni* and/or *P. odocoilei*.

INTRODUCTION

Deer of the genus *Odocoileus* are the only reported hosts of the metastrongyloid nematodes *Parelaphostrongylus andersoni* Prestwood 1972 and *P. odocoilei* (Hobmaier and Hobmaier 1934). *P. andersoni* is reported from white-tailed deer (*O. virginianus*) (Prestwood 1972, Pybus and Samuel 1981) while *P. odocoilei* is reported from black-tailed deer (*O. hemionus columbianus*) and mule deer (*O. h. hemionus*) (Hobmaier and Hobmaier 1934, Brunetti 1969, Platt and Samuel 1978). Both parasites have been implicated as direct or indirect mortality factors in individual hosts (Brunetti 1969, Nettles and Prestwood 1976, Chapter IV and V) and the potential exists for their influence on host populations (Chapter IV and V).

In order to investigate the host-parasite relationships between these worms and their hosts, an attempt was made to find a suitable laboratory host for experimental uses. Information gained from such a host could then be used to provide insight into the relationships with the cervid hosts. The use of small, economical, and readily-accessible

laboratory hosts would also avoid many of the expensive and time-consuming problems currently associated with obtaining, raising, and handling neonatal deer fawns.

Previous attempts to find suitable laboratory hosts for species of *Parelaphostrongylus* are restricted to experimental infections of *P. tenuis* in cottontail rabbits (*Sylvilagus floridanus*) (Nettles and Prestwood 1979) and guinea pigs (*Cavia porcellus*) (Anderson and Strelive 1966, Spratt and Anderson 1968) and a mixed inoculum of *P. andersoni* and *P. tenuis* in domestic rabbits (*Oryctolagus cuniculus*) (Nettles and Prestwood 1979). A few *P. tenuis* larvae appear to follow a migration in guinea pigs similar to that reported in white-tailed deer (Spratt and Anderson, 1968). However, *P. tenuis* larvae develop in neural tissue (Anderson 1963) and before reaching patency, the guinea pigs died as a result of damage to the central nervous system (CNS). Neurologic damage has not been described in any infections of *P. andersoni* or *P. odocoilei*. Thus, guinea pigs may provide suitable conditions for maturation of these two species.

MATERIALS AND METHODS

During 1979-1981, 20 juvenile guinea pigs and eight domestic rabbits were each exposed to 100 or 200 third-stage larvae of *P. andersoni* or *P. odocoilei* (see Table I and II). Guinea pigs were lightly anaesthetized with ether prior to the introduction of larvae into the stomach via a small

TABLE I. Recovery of larvae from guinea pigs exposed to Parelaphostronglus spp.

				P. andersoni					
No.'	Dose	Total recovery*	dPE+	Stomach wall	Mesentery	Liver	Diaphragm	Pleural cavity	Lungs	Inter-costals
17	200	0	42							
19	100	0	42							
20	200	2	57		25	50		25		
18	100	2	91			100				
				P. odocoilei						
1	100	12	2	8	92					
3	100	3	4		100					
4	100	8	7		25	75				
6	100	4	9			100				
7	100	10	11		30	70				
8	100	26	36		27			15	58	
14	200	14	45			7	14	7	72	

TABLE I. (continued)

No.'	Dose	Total recovery*	dPE+	Stomach wall	Mesentery	Liver	Diaphragm	Pleural cavity	Lungs	Inter-costals
11	100	16	58			19	6	31	44	
5	100	0	59							
10	100	15	63			8	46		46	
2	100	10	63						100	
9	100	17	70	6		23	12	12	47	
15	200	23	70	2		44	27		22	5
12	100	21	71			38	10		52	
13	200	18	71		17	9	34		40	
16	200	24	72		11	6	6		56	21

' Animals 1-8 treated with cortisone acetate with no apparent effect.

* Percent of dose (Note all larvae recovered after 36 days were encapsulated).

+ Days post exposure.

TABLE II. Recovery of encapsulated larvae (as % per individual host) from rabbits exposed to Parelaphostrongylus spp.

				P. andersoni					
No.	Dose	Total recovery*	dPE+	Stomach	Caecum	Mesentery	Diaphragm	Liver	Lungs
1	100	9	17	44	56				
2	200	9	35	12	82			6	
3	200	0	42						
4	100	1	56		100				
				P. odocoilei					
7	100	16	43			19		44	37
5	200	4	72			29	28		43
6	200	0	133						
8	100	0	250						

* Percent of dose.

+ Days post exposure.

gauge catheter. Rabbits were exposed similarly but were not anaesthetized. Faecal samples from those individuals remaining after 20 days were collected at 23 and 30 days post exposure (dPE) and then daily starting at 35 dPE. Samples were examined using the Baermann technique. Three guinea pigs and three rabbits served as control animals and were treated similarly excluding exposure to larvae. All animals were monitored for clinical signs throughout the infection.

Animals were killed at various times after exposure to worms. The peritoneal and pleural cavities were opened independently and flushed with saline. The viscera were removed, separated, soaked in individual bowls of saline for four to six hours and then examined at 6X magnification. The liver and mesenteries were pressed between two glass plates and examined at 6X magnification. Particular attention was given to the connective tissues in the dorsal peritoneal cavity. The CNS was removed, separated into sacral, lumbar, thoraco-cervical, and cranial regions. It was then shredded, left overnight in saline, and examined at 12X magnification. The vertebral canal was flooded with saline and examined at 12X magnification. The lungs were shredded and then placed overnight in a pepsin/HCl digest at 37C. All skeletal muscles were shredded and examined at 12X magnification. Any lesions noted were either removed and examined at higher magnifications or fixed in 10% buffered formalin for histologic examination.

Three domestic goats were each exposed to 300 (one animal) or 1000 (two animals) third-stage larvae of *P. odocoilei*. Faecal samples for Baermann examination were collected daily for at least three weeks prior to exposure and from 35 dPE until 101 dPE. Animals were monitored for clinical signs throughout the infection.

RESULTS

Guinea pigs

Recovery (expressed as percent of the initial dose) was markedly higher in the 16 animals exposed to *P. odocoilei* ($\bar{x}=14\pm8$) than the four exposed to *P. andersoni* ($\bar{x}=1\pm1$) (Table I).

A pattern of dispersal of larvae was apparent in guinea pigs exposed to *P. odocoilei* (Table I). Initially (2 and 4 dPE), larvae were found only in the stomach wall and the mesenteries. From 7 to 11 dPE, live larvae were present in the mesenteries and the liver. After 11 days, larvae were present in the diaphragm and pleural cavity. No further dispersal was noted. All larvae recovered from the tissues were similar in size and developmental stage to those used in the initial exposure.

Gross lesions also differed throughout the infections. Early in the infections (2 to 4 dPE), a few pinpoint haemorrhages were present in the serosa of the stomach and caecum. Larvae were surrounded by a thin sheath of cells adhered to the cuticle.

After 7 dPE, all animals had thin white tracks in the serosa and subserosa of the liver. Live larvae were found in the parenchyma adjacent to these lesions. All larvae were associated with a local accumulation of cells. Larvae observed after 11 dPE were partially or completely encapsulated in a thin layer of connective tissue. Some of these larvae were dead.

After 36 dPE, the major lesions consisted of small (1.5-2 mm) round white capsules present in connective tissues and on serosal surfaces of a variety of tissues and organs. Capsules on the serosa extended into the underlying parenchyma and contained amorphous necrotic debris. However, the remains of nematode larvae often could be identified among the debris. A few live larvae were also recovered. No evidence of growth or moulting was apparent in these larvae. Live larvae were not recovered after 59 dPE.

Fibrinous pleuritis was present in most animals. The lesions varied from localized small fibrinous tags or thin sheets to extensive fibrinous adhesions among all of the lobes of the lungs and extending to the pleural wall. Small white capsules (as described previously) were often present in the fibrinous tissue and adhered to the lungs and intercostal muscles. Occasionally, raised, hard, yellow-white nodules (3X3 mm) were seen within the pulmonary parenchyma. The contents of these nodules were similar to those within the small capsules.

In histologic sections, granulomatous encapsulation of larvae was noted in the parenchyma and at the serosa of the lungs and liver. Capsules consisted of a core of necrotic cells, nuclear debris, and larvae surrounded by loose connective tissue. Eosinophils and plasma cells were common at the periphery. Adjacent cells were pale and vacuolated. In later infections larvae appeared degenerate.

In the lungs, large lymphocytes, eosinophils, and a few giant cells were common in the alveolar walls and spaces. Atelectasis of tissue adjacent to the granulomas was noted. Lymphoid hyperplasia was seen throughout the lung sections. Bronchi and bronchioles were often occluded with mucous and cellular debris. Emphysema, haemorrhage, and congestion were minor insults.

Localized focal hepatitis was present in portal regions of the liver. Vascular elements in the portal triads throughout the tissue sections were cuffed with large mononuclear cells and a few eosinophils.

In animals exposed to *P. andersoni*, larvae were recovered only from the two later infections (57 and 91 dPE). Gross and histologic lesions were similar to those in guinea pigs exposed to *P. odocoilei*.

Faecal samples of all animals were negative for first-stage larvae of *Parelaphostrongylus* spp. No control animals exhibited any evidence of infection with *P. andersoni* or *P. odocoilei*.

Rabbits

The percent of the dose recovered from rabbits exposed to either *P. andersoni* or *P. odocoilei* was zero in three animals, low in two animals (1,4) and moderate in three ($\bar{x}=14\pm4$)(Table II). Only animals killed early in the infection (<44 dPE) harboured moderate numbers of larvae. In *P. andersoni* infections, these worms were found in the walls of the stomach and caecum. A few larvae were recovered from the liver of one animal. In *P. odocoilei* infections, the larvae were more widely distributed and found in the mesenteries, liver, diaphragm, and lungs. Recovery was zero in three of five rabbits examined after 42 dPE.

No larvae or evidence of infection was seen in control animals.

Goats

Larvae of *P. odocoilei* were unable to establish a patent infection in any of the three goats exposed to worms. Clinical signs were not apparent. No further investigation of these animals was carried out.

DISCUSSION

It is readily apparent that the species examined in this study were not suitable definitive hosts for *Parelaphostrongylus* spp. Recovery rates indicated that *P. odocoilei* was more successful than *P. andersoni* at penetrating and leaving the gut in guinea pigs. However, in

all cases, larvae were overcome by a local host immune response.

The actual route of migration within the hosts is unknown. Results suggested that some larvae moved through the mesenteries to the liver. The hepatic lesions seen in this study suggested that some larvae entered the liver from the serosal surface. However, other larvae appeared to reach the parenchyma via a blood-borne route through the hepatic portal system. Some larvae apparently penetrated the diaphragm in order to reach the pleural cavity but were subsequently trapped in the pleural connective tissues or the fibrinous exudate associated with an inflammation of the lungs. Thus, it appears that larvae migrated in the circulatory system and by a direct movement of larvae through the tissues.

Caution must be used in evaluating the reported data as dosage levels may be such that the normal pattern of migration is obscured. However, distinct patterns of dispersal were apparent. This information may provide insight into further investigation in a host more susceptible to infection.

Results in the current study are remarkably similar to those reported in experimental infections of *P. tenuis* in guinea pigs (Spratt and Anderson 1968). These authors also report larval penetration of the stomach wall and dispersal within the liver and tissues of the peritoneal cavity. Some larvae apparently penetrated the diaphragm and moved through

the lungs and pleural connective tissues. However, in some *P. tenuis* infections, a few larvae successfully migrated to the normal definitive site (the CNS) and developed to the adult stage. In contrast, in the current study, no larvae were able to complete the migration to the skeletal muscles of the host.

The recovery rates of *P. andersoni* and *P. odocoilei* were much lower in rabbits than in guinea pigs. In rabbits, very few larvae were successful in penetrating the gut wall. They exhibited a dispersal pattern similar to that in guinea pigs but were rapidly destroyed. Thus, rabbits appear to be more resistant to these parasite species.

Nettles and Prestwood (1979) report that experimentally-infected domestic rabbits were mildly susceptible to *P. tenuis* while cottontail rabbits were resistant to infection. Although the inoculum given to domestic rabbits contained larvae of *P. andersoni*, no mention is made of whether or not they established an infection. Thus, no patent infections of *Parelaphostrongylus* spp. in rabbits have been reported. Although rabbits are sympatric with deer infected with these parasites, it is unlikely that they play any role in the epizootiology of the worms.

Species of *Parelaphostrongylus* appear to be relatively host specific in terms of the definitive hosts in which they are capable of developing a patent infection. Successful natural and experimental infections are currently restricted

to members of the Cervidae and Bovidae.

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LITERATURE CITED

Anderson, R.C. 1963. The incidence, development, and experimental transmission of *Pneumostrongylus tenuis* Dougherty (Metastongyloidea: Protostrongylidae) of the meninges of the white-tailed deer (*Odocoileus virginianus borealis*) in Ontario. Can. J. Zool. 41: 775-792.

Anderson, R. C., and U. R. Strelive. 1966. The transmission of *Pneumostrongylus tenuis* to guinea pigs. Can. J. Zool. 44: 533-540.

Brunetti, O. A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game 55: 307-316.

Hobmaier, A., and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n. sp., a new lungworm in black tail deer (*Odocoileus columbianus*). Description and life history.

Proc. Soc. Exp. Biol. Med. 31: 509-514.

Nettles, V. F., and A. K. Prestwood. 1976. Experimental *Parelaphostrongylus andersoni* infections in white-tailed deer. Vet. Pathol. 13: 381-393.

__, 1979. Experimental infection of rabbits with meningeal worm. J. Parasitol. 65: 327-328.

Platt, T. R. and W. M. Samuel. 1978. A redescription and neotype designation for *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea). J. Parasitol. 64: 226-232.

Prestwood, A. K. 1972. *Parelaphostrongylus andersoni* sp. n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus virginianus*). J. Parasitol. 48: 897-902.

Pybus, M. J. and W. M. Samuel. 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Manage. 45: 537-542.

Spratt, D. M. and R. C. Anderson. 1968. The guineapig as an experimental host of the meningeal worm, *Parelaphostrongylus tenuis* Dougherty. J. Helminthol. 42: 139-156.

X. Appendix IV: A case report of two mule deer fawns exposed to 20 third-stage larvae of *P. odocoilei*.

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INTRODUCTION

As part of a project not directly related to this thesis, neonatal fawns were exposed to low levels of *Parelaphostrongylus odocoilei*. Two mule deer (*Odocoileus hemionus hemionus*) were killed and the carcasses searched for adult worms. This report presents the necropsy results of these two deer.

MATERIALS AND METHODS

Two MD fawns were received at the University of Alberta, Biomedical Animal Centre, Ellerslie, Alberta on June 11, 1982. and maintained as outlined in Chapter II. On June 12, the fawns were exposed *per os* to ten third-stage larvae of *P. odocoilei*. On June 13 and 14, they each received an additional five larvae (a total of 20 larvae over three days).

In general, subsequent methods were identical to those detailed in Chapter III. Briefly, daily faecal samples for analysis with the Baermann technique were collected starting 30 days post exposure (dPE). Animals were killed with sodium pentabarbitol and the skeletal muscles, central nervous system, vertebral canal, and fat depots in the pelvic cavity examined for adults and larvae of *P. odocoilei*.

RESULTS

Prepatent period has not yet been determined. However, both deer were passing dorsal-spined larvae in faeces by 58 dPE. The pattern of larval output has not yet been determined.

Gross pathology observed at necropsy consisted of lesions in the lungs, lymph nodes, and skeletal muscles. The lungs of MD 68 were slightly firm and enlarged with evidence of mild interstitial pneumonia. Seven firm, round, 2mm diameter nodules were present in the diaphragmatic lobes. Lesions were classified in class 4 (see Chapter IV for an explanation of the classification). The lungs of MD 69 contained a few focal haemorrhages and areas of grey discolouration (class 2).

The deep inguinal lymph nodes were enlarged and haemorrhagic in MD 69 but not in MD 68. The mesenteric lymph node of MD 68 was haemorrhagic with discrete soft white areas (1-2mm diameter) in the medulla. These latter areas did not contain any significant bacterial agents.

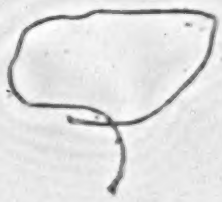
Damage to the skeletal muscles consisted of small areas of haemorrhage (2-5mm diameter) associated with the presence of adult *P. odocoilei*. Nine (45% of the initial dose) and seven (35%) adult worms were recovered from MD 68 and 69, respectively. The worms tended to be in pairs (one male, one female) and were distributed in the backstraps, thighs, and shank (Table 1). Worms were not found in the central nervous system, vertebral canal, or pelvic fat depots.

DISCUSSION

Many aspects of these two infections were similar to those in MD exposed to 300 larvae of *P. odocoilei* (see Chapters III and V). The prepatent period, although not yet precisely identified, appears to be similar to that seen in higher doses. The gross lesions were generally reduced in the deer receiving 20 larvae; however, the lungs of one animal were heavily damaged. The recovery rate and distribution of adult worms were similar to those in deer exposed to 300 larvae and, as in previous experiments, the distribution appeared to change over time.

In conclusion, exposure of MD to a low dose of *P. odocoilei* (20 larvae) did not alter the basic patterns in the host-parasite relationship observed in animals exposed to higher levels (300 larvae).

Table 1. Location of adult Parelaphostrongylus odocoilei in the skeletal muscles of two mule deer (MD) fawns.

Deer No.	Total recovery			No. worms recovered		
	No.	% dose	dPE*	Backstraps	Thighs	Shank
MD 69	7	35	108	3	4	0
MD 68	9	45	151	0	7	2

* Days post exposure.